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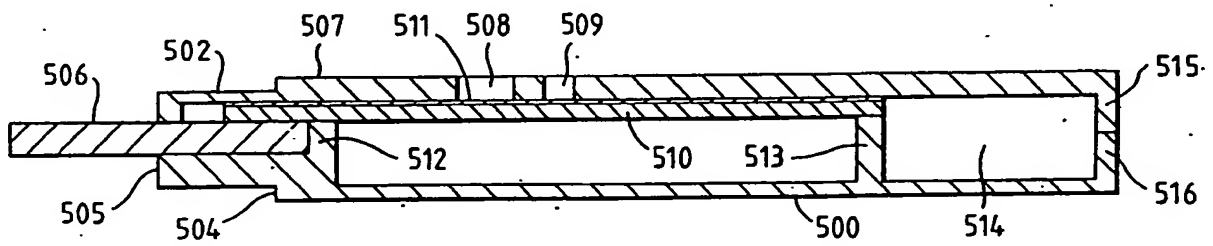

(57) Abstract

An analytical test device useful for example in pregnancy testing, comprises a hollow casing (500) constructed of moisture-impervious solid material, such as plastics materials, containing a dry porous carrier (510) which communicates indirectly with the exterior of the casing via a bibulous sample receiving member (506) which protudes from the casing such that a liquid test sample can be applied to the receiving member and permeate therefrom to the porous carrier, the carrier containing in a first zone a labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilised on the carrier material and is therefore not mobile in the moist state, the two zones being arranged such that liquid sample applied to the porous carrier can permeate via the first zone into the second zone, and the device incorporating means, such as an aperture (508) in the casing, enabling the extent (if any) to whichthe labelled reagent becomes bound in the second zone to be observed. Preferably the device includes a removable cap for the protruding bibulous member.

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Immunoassays and devices therefor.

The present invention relates to assays involving specific binding, especially immunoassays.

In particular, the invention relates to analytical devices which are suitable for use in the home, clinic or doctor's surgery and which are intended to give an analytical result which is rapid and which requires the minimum degree of skill and involvement from the user. The use of test devices in the home to test for pregnancy and fertile period (ovulation) is now commonplace, and a wide variety of test devices and kits are available commercially. Without exception, the commercially-available devices all require the user to perform a sequence of operations before the test result is observable. These operations necessarily involve time, and introduce the possibility of error.

It is an object of the present invention to provide a test device which is readily usable by an unskilled person and which preferably merely requires that some portion of

the device is contacted with the sample (e.g. a urine stream in the case of a pregnancy or ovulation test) and thereafter no further actions are required by the user before an analytical result can be observed. Ideally the analytical result should be observable within a matter of minutes following sample application, e.g. ten minutes or less.

The use of reagent-impregnated test strips in specific binding assays, such as immunoassays, has previously been proposed. In such procedures a sample is applied to one portion of the test strip and is allowed to permeate through the strip material, usually with the aid of an eluting solvent such as water. In so doing, the sample progresses into or through a detection zone in the test strip wherein a specific binding reagent for an analyte suspected of being in the sample is immobilised. Analyte present in the sample can therefore become bound within the detection zone. The extent to which the analyte becomes bound in that zone can be determined with the aid of labelled reagents which can also be incorporated in the test strip or applied thereto subsequently. Examples of prior proposals utilising these principles are given in Thyroid Diagnostics Inc GB 1589234, Boots-Celltech Diagnostics Limited EP 0225054, Syntex (USA) Inc EP 0183442, and Behringwerke AG EP 0186799.

The present invention is concerned with adapting and improving the known techniques, such as those referred to in the above publications, to provide diagnostic test devices especially suitable for home use which are quick and convenient to use and which require the user to perform as few actions as possible.

A typical embodiment of the invention is an analytical test device comprising a hollow casing constructed of moisture-impervious solid material containing a dry porous carrier which communicates directly or indirectly with the exterior of the casing such that a liquid test sample can be applied to the porous carrier, the device also containing a labelled specific binding reagent for an analyte which labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilised in a detection zone on the carrier material and is therefore not mobile in the moist state, the relative positioning of the labelled reagent and detection zone being such that liquid sample applied to the device can pick up labelled reagent and thereafter permeate into the detection zone, and the device incorporating means enabling the extent (if any) to which the labelled reagent becomes in the detection zone to be observed.

Another embodiment of the invention is a device for use in an assay for an analyte, incorporating a porous solid phase material carrying in a first zone a labelled reagent which is retained in the first zone while the porous material is in the dry state but is free to migrate through the porous material when the porous material is moistened, for example by the application of an aqueous liquid sample suspected of containing the analyte, the porous material carrying in a second zone, which is spatially distinct from the first zone, an unlabelled specific binding reagent having specificity for the analyte, and which is capable of participating with the labelled reagent in either a "sandwich" or a "competition" reaction, the unlabelled specific binding reagent being firmly immobilised on the porous material such that it is

not free to migrate when the porous material is in the moist state.

The invention also provides an analytical method in which a device as set forth in the proceeding paragraph is contacted with an aqueous liquid sample suspected of containing the analyte, such that the sample permeates by capillary action through the porous solid phase material via the first zone into the second zone and the labelled reagent migrates therewith from the first zone to the second zone, the presence of analyte in the sample being determined by observing the extent (if any) to which the labelled reagent becomes bound in the second zone.

In one embodiment of the invention, the labelled reagent is a specific binding partner for the analyte. The labelled reagent, the analyte (if present) and the immobilised unlabelled specific binding reagent cooperate together in a "sandwich" reaction. This results in the labelled reagent being bound in the second zone if analyte is present in the sample. The two binding reagents must have specificities for different epitopes on the analyte.

In another embodiment of the invention, the labelled reagent is either the analyte itself which has been conjugated with a label, or is an analyte analogue, ie a chemical entity having the identical specific binding characteristics as the analyte, and which similarly has been conjugated with a label. In the latter case, it is preferable that the properties of the analyte analogue which influence its solubility or dispersibility in an aqueous liquid sample and its ability to migrate through the moist porous solid phase material should be identical to those of the analyte itself, or at least very closely similar. In this second embodiment, the labelled analyte or analyte analogue will migrate through the porous solid

phase material into the second zone and bind with the immobolised reagent. Any analyte present in the sample will compete with the labelled reagent in this binding reaction. Such competition will result in a reduction in the amount of labelled reagent binding in the second zone, and a consequent decrease in the intensity of the signal observed in the second zone in comparison with the signal that is observed in the absence of analyte in the sample.

An important preferred embodiment of the invention is the selection of nitrocellulose as the carrier material. This has considerable advantage over conventional strip materials, such as paper, because it has a natural ability to bind proteins without requiring prior sensitisation. Specific binding reagents, such as immunoglobulins, can be applied directly to nitrocellulose and immobilised thereon. No chemical treatment is required which might interfere with the essential specific binding activity of the reagent. Unused binding sites on the nitrocellulose can thereafter be blocked using simple materials, such as polyvinylalcohol. Moreover, nitrocellulose is readily available in a range of pore sizes and this facilitates the selection of a carrier material to suit particularly requirements such as sample flow rate.

Another important preferred embodiment of the invention is the use of so called "direct labels", attached to one of the specific binding reagents. Direct labels such as gold sols and dye sols, are already known per se. They can be used to produce an instant analytical result without the need to add further reagents in order to develop a detectable signal. They are robust and stable and can therefore be used readily in a analytical device which is stored in the dry state. Their release on contact with an aqueous sample can be modulated, for example by the use of soluble glazes.

An important aspect of the invention is the selection of technical features which enable a direct labelled specific binding reagent to be used in a carrier-based analytical device, e.g. one based on a strip format, to give a quick and clear result. Ideally, the result of the assay should be discernable by eye and to facilitate this, it is necessary for the direct label to become concentrated in the detection zone. To achieve this, the direct labelled reagent should be transportable easily and rapidly by the developing liquid. Furthermore, it is preferable that the whole of the developing sample liquid is directed through a comparatively small detection zone in order that the probability of an observable result being obtained in increased.

Another important aspect of the invention is the use of a directly labelled specific binding reagent on a carrier material comprising nitrocellulose. Preferably the nitrocellulose has a pore size of at least one micron. Preferably the nitrocellulose has a pore size not greater than about 20 microns. In a particularly preferred embodiment, the direct label is a coloured latex particle of spherical or near-spherical shape and having a maximum diameter of not greater than about 0.5 micron. An ideal size range for such particles is from about 0.05 to about 0.5 microns.

In a further embodiment of the present invention, the porous solid phase material is linked to a porous receiving member to which the liquid sample can be applied and from which the sample can permeate into the porous solid phase material. Preferably, the porous solid phase material is contained within a moisture-impermeable casing or housing and the porous receiving member, with which the porous solid phase material is linked, extends out of the housing and can act as a means for permitting a liquid

sample to enter the housing and permeate the porous solid phase material. The housing should be provided with means, e.g. appropriately placed apertures, which enable the second zone of the porous solid phase material (carrying the immobilised unlabelled specific binding reagent) to be observable from outside the housing so that the result of the assay can be observed. If desired, the housing may also be provided with further means which enable a further zone of the porous solid phase material to be observed from outside the housing and which further zone incorporates control reagents which enable an indication to be given as to whether the assay procedure has been completed. Preferably the housing is provided with a removable cap or shroud which can protect the protruding porous receiving member during storage before use. If desired, the cap or shroud can be replaced over the protruding porous receiving member, after sample application, while the assay procedure is being performed. Optionally, the labelled reagent can be incorporated elsewhere within the device, e.g. in the bibulous sample collection member, but his is not preferred.

An important embodiment of the invention is a pregnancy testing device comprising a hollow elongated casing containing a dry porous nitrocellulose carrier which communicates indirectly with the exterior of the casing via a bibulous urine receiving member which protrudes from the casing and which can act as a reservoir from which urine is released into the porous carrier, the carrier containing in a first zone a highly-specific anti-hCG antibody bearing a coloured "direct" label, the labelled antibody being freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone an highly-specific unlabelled anti-hCG antibody which is permanently immobilised on the carrier material and is therefore not

mobile in the moist state, the labelled and unlabelled antibodies having specificities for different hCG epitopes, the two zones being arranged such that a urine sample applied to the porous carrier can permeate via the first zone into the second zone, and the casing being constructed of opaque or translucent material incorporating at least one aperture through which the analytical result may be observed, together with a removable and replaceable cover for the protruding bibulous urine receiving member. A fertile period prediction device, essentially as just defined except that the analyte is LH, is an important alternative.

Such devices can be provided as kits suitable for home use, comprising a plurality (e.g. two) of devices individually wrapped in moisture impervious wrapping and packaged together with appropriate instructions to the user.

The porous sample receiving member can be made from any bibulous, porous or fibrous material capable of absorbing liquid rapidly. The porosity of the material can be unidirectional (ie with pores or fibres running wholly or predominantly parallel to an axis of the member) or multidirectional (omnidirectional, so that the member has an amorphous sponge-like structure). Porous plastics material, such as polypropylene, polyethylene (preferably of very high molecular weight), polyvinylidene flouride, ethylene vinylacetate, acrylonitrile and polytetrafluoro-ethylene can be used. It can be advantageous to pre-treat the member with a surface-active agent during manufacture, as this can reduce any inherent hydrophobicity in the member and therefore enhance its ability to take up and deliver a moist sample rapidly and efficiently. Porous sample receiving members can also be made from paper or other cellulosic materials, such as

nitro-cellulose. Materials that are now used in the nibs of so-called fibre tipped pens are particularly suitable and such materials can be shaped or extruded in a variety of lengths and cross-sections appropriate in the context of the invention. Preferably the material comprising the porous receiving member should be chosen such that the porous member can be saturated with aqueous liquid within a matter of seconds. Preferably the material remains robust when moist, and for this reason paper and similar materials are less preferred in any embodiment wherein the porous receiving member protrudes from a housing. The liquid must thereafter permeate freely from the porous sample receiving member into the porous solid phase material.

If present, the "control" zone can be designed merely to convey an unrelated signal to the user that the device has worked. For example, the control zone can be loaded with an antibody that will bind to the labelled antibody from the first zone, e.g. an "anti-mouse" antibody if the labelled body is one that has been derived using a murine hybridoma, to confirm that the sample has permeated the test strip. Alternatively, the control zone can contain an anhydrous reagent that, when moistened, produces a colour change or colour formation, e.g. anhydrous copper sulphate which will turn blue when moistened by an aqueous sample. As a further alternative, a control zone could contain immobilised analyte which will react with excess labelled reagent from the first zone. As the purpose of the control zone is to indicate to the user that the test has been completed, the control zone should be located downstream from the second zone in which the desired test result is recorded. A positive control indicator therefore tells the user that the sample has permeated the required distance through the test device.

The label can be any entity the presence of which can be readily detected. Preferably the label is a direct label, ie an entity which, in its natural state, is readily visible either to the naked eye, or with the aid of an optical filter and/or applied stimulation, e.g. UV light to promote fluorescence. For example, minute coloured particles, such as dye sols, metallic sols (e.g. gold), and coloured latex particles, are very suitable. Of these options, coloured latex particles are most preferred. Concentration of the label into a small zone or volume should give rise to a readily detectable signal, e.g. a strongly-coloured area. This can be evaluated by eye, or by instruments if desired.

Indirect labels, such as enzymes, e.g. alkaline phosphatase and horse radish peroxidase, can be used but these usually require the addition of one or more developing reagents such as substrates before a visible signal can be detected. Hence these are less preferred. Such additional reagents can be incorporated in the porous solid phase material or in the sample receiving member, if present, such that they dissolve or disperse in the aqueous liquid sample. Alternatively, the developing reagents can be added to the sample before contact with the porous material or the porous material can be exposed to the developing reagents after the binding reaction has taken place.

Coupling of the label to the specific binding reagent can be by covalent bonding, if desired, or by hydrophobic bonding. Such techniques are commonplace in the art, and form no part of the present invention. In the preferred embodiment, where the label is a direct label such as a coloured latex particle, hydrophobic bonding is preferred.

In all embodiments of the invention, it is essential that the labelled reagent migrates with the liquid sample as this progresses to the detection zone. Preferably, the flow of sample continues beyond the detection zone and sufficient sample is applied to the porous material in order that this may occur and that any excess labelled reagent from the first zone which does not participate in any binding reaction in the second zone is flushed away from the detection zone by this continuing flow. If desired, an absorbant "sink" can be provided at the distal end of the carrier material. The absorbent sink may comprise of, for example, Whatman 3MM chromatography paper, and should provide sufficient absorptive capacity to allow any unbound conjugage to wash out of the detection zone. As an alternative to such a sink it can be sufficient to have a length of porous solid phase material which extends beyond the detection zone.

The presence or intensity of the signal from the label which becomes bound in the second zone can provide a qualitative or quantitative measurement of analyte in the sample. A plurality of detection zones arranged in series on the porous solid phase material, through which the aqueous liquid sample can pass progressively, can also be used to provide a quantitative measurement of the analyte, or can be loaded individually with different specific binding agents to provide a multi-analyte test.

The immobilised specific binding reagent in the second zone is preferably a highly specific antibody, and more preferably a monoclonal antibody. In the embodiment of the invention involving the sandwich reaction, the labelled reagent is also preferably a highly specific antibody, and more preferably a monoclonal antibody.

Preferably the carrier material is in the form of a strip or sheet to which the reagents are applied in spacially distinct zones, and the liquid sample is allowed to permeate through the sheet or strip from one side or end to another.

If desired, a device according to the invention can incorporate two or more discrete bodies of porous solid phase material, e.g. separate strips or sheets, each carrying mobile and immobilised reagents. These discrete bodies can be arranged in parallel, for example, such that a single application of liquid sample to the device initiates sample flow in the discrete bodies simultaneously. The separate analytical results that can be determined in this way can be used as control results, or if different reagents are used on the different carriers, the simultaneous determination of a plurality of analytes in a single sample can be made. Alternatively, multiple samples can be applied individually to an array of carriers and analysed simultaneously.

The material comprising the porous solid phase is preferably nitrocellulose. This has the advantage that the antibody in the second zone can be immobilised firmly without prior chemical treatment. If the porous solid phase material comprises paper, for example, the immobilisation of the antibody in the second zone needs to be performed by chemical coupling using, for example, CNBr, carbonyldiimidazole, or tresyl chloride.

Following the application of the antibody to the detection zone, the remainder of the porous solid phase material should be treated to block any remaining binding sites elsewhere. Blocking can be achieved by treatment with protein (e.g. bovine serum albumin or milk protein), or with polyvinylalcohol or ethanolamine, or any

combination of these agents, for example. The labelled reagent for the first zone can then be dispensed onto the dry carrier and will become mobile in the carrier when in the moist state. Between each of these various process steps (sensitisation, application of unlabelled reagent, blocking and application of the labelled reagent), the porous solid phase material should be dried.

To assist the free mobility of the labelled reagent when the porous carrier is moistened with the sample, it is preferable for the labelled reagent to be applied to the carrier as a surface layer, rather than being impregnated in the thickness of the carrier. This can minimise interaction between the carrier material and the labelled reagent. In a preferred embodiment of the invention, the carrier is pre-treated with a glazing material in the region to which the labelled reagent is to be applied. Glazing can be achieved, for example, by depositing an aqueous sugar or cellulose solution, e.g. of sucrose or lactose, on the carrier at the relevant portion, and drying. The labelled reagent can then be applied to the glazed portion. The remainder of the carrier material should not be glazed.

Preferably the porous solid phase material is nitrocellulose sheet having a pore size of at least about 1 micron, even more preferably of greater than about 5 microns, and yet more preferably about 8-12 microns. Very suitable nitrocellulose sheet having a nominal pore size of up to approximately 12 microns, is available commercially from Schleicher and Schuell GmbH.

Preferably, the nitrocellulose sheet is "backed", e.g. with plastics sheet, to increase its handling strenth. This can be manufactured easily by forming a thin layer of nitrocellulose on a sheet of backing

material. The actual pore size of the nitrocellulose when backed in this manner will tend to be, lower than that of the corresponding unbacked material.

Alternatively, a pre-formed sheet of nitrocellulose can be tightly sandwiched between two supporting sheets of solid material, e.g. plastics sheets.

It is preferable that the flow rate of an aqueous sample through the porous solid phase material should be such that in the untreated material, aqueous liquid migrates at a rate of 1cm in not more than 2 minutes, but slower flow rates can be used if desired.

The spatial separation between the zones, and the flow rate characteristics of the porous carrier material, can be selected to allow adequate reaction times during which the necessary specific binding can occur, and to allow the labelled reagent in the first zone to dissolve or disperse in the liquid sample and migrate through the carrier. Further control over these parameters can be achieved by the incorporation of viscosity modifiers (e.g. sugars and modified celluloses) in the sample to slow down the reagent migration.

Preferably, the immobilised reagent in the second zone is impregnated throughout the thickness of the carrier in the second zone (e.g. throughout the thickness of the sheet or strip if the carrier is in this form). Such impregnation can enhance the extent to which the immobilised reagent can capture any analyte present in the migrating sample.

The reagents can be applied to the carrier material in a variety of ways. Various "printing" techniques have previously been proposed for application of liquid

reagents to carriers, e.g. micro-syringes, pens using metered pumps, direct printing and ink-jet printing, and any of these techniques can be used in the present context. To facilitate manufacture, the carrier (e.g. sheet) can be treated with the reagents and then subdivided into smaller portions (e.g. small narrow strips each embodying the required reagent-containing zones) to provide a plurality of identical carrier units.

By way of example only, some preferred embodiments of the invention will now be described in detail with reference to the accompanying drawings.

Embodiment 1

Figures 1 and 2 represent a typical strip of porous solid phase material for use in an assay test in accordance with the invention, and illustrate the underlying principle upon which the invention operates.

Referring to Figure 1, the assay test strip 10 is seen as a rectangular strip having (for the purpose of this description) its longitudinal axis in a vertical situation. Adjacent the lower end 11 of strip 10 is a narrow band or zone 12 extending across the entire width of the strip. A small region 13 of strip 10 lies vertically below zone 12. Above zone 12 is a second zone 14 lying a discrete distance up strip 10 and similarly extending the entire width of the strip. The region 15 of strip 10 between zones 12 and 14 can be of any height as long as the two zones are separate. A further region 16 of the strip extends above zone 14, and at the top 17 of the strip is a porous pad 18 firmly linked to strip 10 such that pad 18 can act as a "sink" for any liquid sample which may be rising by capillary action through strip 10.

Zone 12 is loaded with a first antibody bearing a visible ("direct") label (e.g. coloured latex particle, dye sol or gold sol). This reagent can freely migrate through the strip in the presence of a liquid sample. In zone 14, the strip is impregnated with a second antibody having specificity for a different epitope on the same analyte as the first antibody. The second antibody is firmly immobilised on the strip.

Figure 2 illustrates what happens when the assay strip is used in an analytical procedure. The lower end 11 of the dry strip is contacted with a liquid sample (not shown) which may contain the analyte to be determined. Capillary action causes the fluid to rise through the strip and eventually reach pad 18. In so doing, the sample traverses zone 12 and the labelled antibody will dissolve or disperse in the sample and migrate with it through the strip. While migrating towards zone 14, the labelled antibody can bind to any analyte present in the sample. On reaching zone 14, any analyte molecule should become bound to the second antibody, so immobilising the labelled "sandwich" so produced. If a significant concentration of the analyte to be determined is present in the liquid sample, in a short period of time a distinct accumulation of the visible label should occur in zone 14.

As an example of an analysis to which this embodiment can be applied, the analyte can be hCG, the reagents in zones 12 and 14 can be monoclonal antibodies to hCG which can participate in a "sandwich" reaction with hCG, and the label can be a particulate dye, a gold sol or coloured latex particles.

Although described above in relation to a "sandwich" reaction, it will be readily apparent to the skilled reader that this can be modified to a "competition"

reaction format if desired, the labelled reagent in zone 12 being the analyte or an analogue of the analyte.

An assay based on the above principles can be used to determine a wide variety of analytes by choice of appropriate specific binding reagents. The analytes can be, for example, proteins, haptens, immunoglobulins, hormones, polyneucleotides, steroids, drugs, infectious disease agents (e.g. of bacterial or viral origin) such as Streptoccus, Neisseria and Chlamydia. Sandwich assays, for example, may be performed for analytes such as hCG, LH, and infectious disease agents, whereas competition assays, for example, may be carried out for analytes such as E-3-G and P-3-G.

The determination of the presence (if any) of more than one analyte in sample can have significant clinical utility. For example, the ratio of the levels of apolipoproteins A_1 and B can be indicative of susceptibility to coronary heart disease. Similarly, the ratio of the levels of glycated haemoglobin (HbA) to unglycated (HbAo) or total (Hb) haemoglobin can aid in the management of diabetes. Additionally it is possible to configure tests to measure two steroids simultaneously, e.g E-3-G and P-3-G. By way of example, a dual analyte test for apolipoproteins A_1 and B may be prepared by depositing, as two spacially distinct zones, antibody specific for apolipoprotein A_1 throughout a first zone and depositing a second antibody specific for apolipoprotein B, throughout the second zone of a porous carrier matrix. Following the application of both antibodies to each of their respective zones via a suitable application procedure (e.g. ink-jet printing, metered pump and pen, or airbrush), the remainder of the porous material should be treated with a reagent, e.g. bovine serum albumin, polyvinyl alcohol, or ethanolamine, to block any remaining

binding sites elsewhere. A third and fourth reagent, bearing a label, may then be dispensed onto the dry carrier in one or more zones near to one end of the strip, the strip being allowed to dry between applications of the two reagents to the same zone. Reagent 3 and Reagent 4 may comprise conjugates of anti-apolipoprotein A_1 antibody and anti-apolipoprotein B antibody respectively. Both of these conjugates will become mobile in and on the carrier when in the moist state. Reagents 3 and 4 can migrate with the solvent flow when an aqueous sample is applied to the first end of the carrier strip. While migrating towards the two zones further along the strip, reagent 3 may bind any apolipoprotein A_1 present in the sample and reagent 4 may bind any apolipoprotein B present in the sample. On reaching the first second-antibody zone (anti-apolipoprotein A_1 antibody zone) anti-apolipoprotein A_1 molecules should become bound to the second antibody, immobilising the labelled 'sandwich' so produced. No labelled apoliprotein B molecules will bind to this first zone. On reaching the second second-antibody zone (anti-apolipoprotein B antibody zone) any apolipoprotein B molecules should become bound to the second antibody (solid-phase antibody), immobilising the labelled 'sandwich' so produced. No labelled apolipoprotein A_1 molecules will bind to the second zone. An accumulation of each of the direct label may occur at both or either zones to a lesser or greater extent resulting in a visible signal at either or both of the solid phase antibody zones. Excess unbound conjugate (of both reagent 3 and reagent 4) can pass freely over the two antibody zones and will be washed into the distal end of the strip.

The development of a quantifiable colour in both of the second-antibody zones may be assessed with an appropriate form of instrumentation, yielding a ratio of colour density between the two sites.

The determination of the presence of more than two (ie multiple) analytes in any sample may have significant clinical utility. For example, the detection of the presence of various different serotypes of one bacterium, or the detection of the presence of soluble serological markers in humans may be useful. By way of example, a multiple analyte test for the detection of the presence of different serotypes of Streptococcus can be prepared for groups A, B, C and D. A cocktail of monoclonal antibodies, each specific for various pathologically important group serotypes, or a polyclonal antiserum raised against a particular Streptococcal group, can be dispensed onto a porous carrier strip as a line extending the width of the strip of approximately 1mm zone length. Multiple lines be dispensed in spatially discrete zones, each zone containing immunochemically reactive component(s) capable of binding the analyte of interest. Following the application of the multiple zones, via a suitable application procedure (eg ink-jet printing, metered pump and pen, airbrush), the remainder of the porous material should be treated with a reagent (eg bovine serum albumin, polyvinylalcohol, ethanolamine) to block any remaining binding sites elsewhere. Conjugates of label, e.g. a dye sol, and each immunochemically-reactive component specific for each bacterial group may then be dispensed either onto a single zone at the bottom end of the strip, proximal to the sample application zone, or as a series of separate zones.

Figures 3, 4 and 5 of the accompanying drawings depict a complete device utilising a porous strip as just described above. Figure 3 represents the complete device viewed from the front, Figure 4 shows the same device partially cut away to reveal the details of the strip inside, and Figure 5 shows the underside of the device.

Referring to Figure 3, the device comprises a flat rectangular body 30 the front face 31 of which is perforated by a circular hole or window 32 which reveals the porous test strip 10 within the body. The region of the test strip 10 visible through the window 32 incorporates a narrow horizontal zone 14.

Referring to Figure 4, the device comprises a dry rectangular test strip 10 made from porous material which extends from the bottom end 33 of the body 30 within the body between the front 31 and back 34 of the body. Near the bottom end 11 of the strip 10 is a horizontal zone 12 bearing a labelled specific binding reagent for an analyte, the binding reagent being mobile in the test strip in the moist state. Further up the test strip is the narrow horizontal zone 14 which is visible through the window 32. At the top 17 of the test strip 10 is a porous 'sink' 18 which can absorb any liquid sample that has permeated upwards through the strip.

Referring to Figure 5, the bottom edge 35 of the body 30 incorporates a lateral aperture in which the bottom end 11 of the strip lies.

In operation, the bottom end 33 of the body 30 is immersed in a liquid sample (eg urine) so that the liquid sample can be absorbed by the bottom end 11 of the test strip 20 and rise by capillary action to the top 17 of the test strip and into the sink 18. In so doing, the liquid sample progresses via zone 12 to zone 14. Specific binding reactions as described above occur, and the test result is visible to the user through the window 32.

Embodiment 2

Figures 6 and 7 of the accompanying drawings illustrate another test device according to the invention. Figure 6 illustrates the complete device viewed from the front, and Figure 7 depicts the same device partially cut away to reveal details of a porous test strip contained within the body of the device.

Referring to Figure 6, the device comprises an elongate body 200 terminating at its lower end 201 in a small integral receptacle 202 which can hold a predetermined volume of a liquid sample, eg urine. The front face 203 of the body 200 incorporates two square small square apertures or windows 204 and 205 located one above the other.

Referring to Figure 7, the elongate portion of the body 200 is hollow and incorporates a test strip 206 running almost the full height of the body. This test strip is of similar construction to those described under Embodiment 1, and incorporates near its lower end 207 a horizontal zone 208 bearing a labelled specific binding reagent that can freely migrate in the strip in the moist state. There are two circular zones 209 and 210 adjacent to the windows 204 and 205 and visible therethrough. The strip terminates at its top end 211 in a porous sink 212. At the bottom end 201 of the device, the receptacle 202 communicates with the hollow body via a lateral aperture 213.

In operation, a liquid sample is applied to the bottom end of the device and a predetermined volume of the sample fills the receptacle 202. From the receptacle 202 the liquid sample rises by capillary action through the test strip 206 and conveys the labelled reagent from zone 208 to the two circular zones 209 and 210. A series of specific binding reactions as described in relation to

Embodiment 1 above occur. In this embodiment the second circular zone 210 can act as a control (giving rise, for example, to a coloured signal irrespective of whether or not the sample contains the analyte to be determined) and the determination of the analyte takes place in the first circular zone 209. The user can determine whether the analyte is present in the sample by comparing the signal produced in the two zones.

For example, if the test is used to determine the presence of hCG in urine during the course of a pregnancy test, the circular control zone 210 can contain immobilised HCG which will bind a labelled antibody which is carried upwards from zone 208 by the migrating liquid sample. The same labelled antibody can engage in a 'sandwich' reaction with hCG in the sample and be bound in the first circular zone 209 by another specific anti-hCG antibody which has been immobilised therein. Alternatively, if desired, the "control" zone can be designed merely to convey an unrelated signal to the user that the device has worked. For example, the second circular zone can be loaded with an antibody that will bind to the labelled antibody from zone 208, e.g. an "anti-mouse" antibody if the labelled antibody is one that has been derived using a murine hybridoma, to confirm that the sample has permeated the test strip.

Embodiment 3

Figure 8 of the accompanying drawings represents an isometric view of an assay device in accordance with the invention, and Figure 9 represents a cross-sectional side elevation of the device shown in Figure 8.

Referring to Figure 8, the device comprises a housing or casing 500 of elongate rectangular form having at one

end 501 a portion 502 of reduced cross-sectional area. A cap 503 can be fitted onto portion 502 and can abut against the shoulder 504 at end 501 of the housing. Cap 503 is shown separated from housing 500. Extending beyond end 505 of portion 502 is a porous member 506. When cap 503 is fitted onto portion 502 of the housing, it covers porous member 506. Upper face 507 of housing 500 incorporates two apertures 508 and 509.

Referring to Figure 9, it can be seen that housing 500 is of hollow construction. Porous member 506 extends into housing 500 and contacts a strip of porous carrier material 510. Porous member 506 and strip 510 overlap to ensure that there is adequate contact between these two materials and that a liquid sample applied to member 506 can permeate member 506 and progress into strip 510. Strip 510 extends further into housing 500. Strip 510 is "backed" by a supporting strip 511 formed of transparent moisture-impermeable plastics material. Strip 510 extends beyond apertures 508 and 509. Means are provided within housing 500 by webbs 512 and 513 to hold strip 510 firmly in place. In this respect, the internal constructional details of the housing are not a significant aspect of the invention as long as the strip is held firmly in place within the housing, and porous member 506 is firmly retained in the housing and adequate fluid permeable contact is maintained between member 506 and strip 510. The transparent backing strip 511 lies between strip 510 and apertures 508 and 509 and can act as a seal against ingress of moisture from outside the housing 500 via these apertures. If desired, the residual space 514 within the housing can contain moisture-absorbant material, such as silica gel, to help maintain the strip 510 in the dry state during storage. The reagent-containing zones in strip 510 are not depicted in Figure 8, but the first zone containing the labelled reagent which is mobile when the

strip is moistened will lie in the region between the porous member 506 and aperture 508. The second zone containing the immobilised unlabelled reagent will lie in the region exposed through aperture 508 in order that when the device has been used in an assay, the result can be observed through aperture 508. Aperture 509 provides means through which a control zone containing further reagents which may enable the adequate permeation of sample through the strip to be observed.

In operation, the protective cap 503 is removed from the holder and member 506 is exposed to a liquid sample e.g. by being placed in a urine stream in the case of a pregnancy test. After exposing member 506 to the liquid sample for a time sufficient to ensure that member 506 is saturated with the sample, the cap 503 can be replaced and the device placed aside by the user for an appropriate period time (e.g. two or three minutes) while the sample permeates test strip 510 to provide the analytical result. After the appropriate time, the user can observe the test strip through apertures 508 and 509 and can ascertain whether the assay has been completed by observing the control zone through aperture 509, and can ascertain the result of the assay by observing the second zone through aperture 508.

During manufacture, the device can be readily assembled from, for example, plastics material with the housing 500 being moulded in two parts (e.g. upper and lower halves 515 and 516) which can be securely fastened together (e.g. by ultrasonic welding) after the porous member and test strip have been placed within one of the halves and then sandwiched between the two halves. The act of forming this sandwich construction can be used to "crimp" the porous member and test strip together to ensure adequate contact between them. Cap 503 can be

moulded as a separate complete item. If desired, apertures 508 and 509 can be provided with transparent inserts which may insure greater security against ingress of extraneous moisture from outside the housing. By providing a tight fit between the end 505 of housing 500 and the protruding porous member 506, the application of sample to the protruding member will not result in sample entering the device directly and by-passing member 506. Member 506 therefore provides the sole route of access for the sample to the strip within the housing, and can deliver sample to the strip in a controlled manner. The device as a whole therefore combines the functions of samples and analyser.

By using the test strip materials and reagents as hereinafter described, a device in accordance with Figures 8 and 9 can be produced which is eminently suitable for use as a pregnancy test kit or fertile period test kit for use in the home or clinic. The user merely needs to apply a urine sample to the exposed porous member and then (after optionally replacing the cap) can observe the test result through aperture 508 within a matter of a few minutes.

Although described with particular reference to pregnancy tests and fertile period tests, it will be appreciated that the device, as just described, can be used to determine the presence of a very wide variety of analytes if appropriate reagents are incorporated in the test strip. It will be further appreciated that aperture 509 is redundant and may be omitted if the test strip does not contain any control means. Further, the general shape of the housing and cap, both in terms of their length, cross-section and other physical features, can be the subject of considerable variation without departing from the spirit of the invention.

A further option is the omission of the labelled reagent from the test strip, this reagent being added to the sample prior to application of the sample to the test device. Alternatively, the labelled reagent can be contained in the protruding porous member 506.

Figure 10 of the accompanying drawings shows an enlarged view of the porous receiving member and test strip in the device illustrated in Figures 8 and 9.

The porous receiving member 506 is linked to the porous test strip 510, backed by the transparent plastics sheet 511, such that liquid can flow in the direction shown by the arrows through the porous receiving member and into the porous strip. Test zone 517 incorporates the immobilised specific binding reagent, and control zone 518 contains a reagent to indicate that the sample has permeated a sufficient distance along the test strip. A portion of the test strip surface opposite the backing strip 511 and adjacent the porous receiving member 506, carries a glaze 519 on which is deposited a layer 520 of labelled specific binding reagent. The thickness of these two layers as depicted in Figure 10 is grossly exaggerated purely for the purpose of illustration. It will be appreciated that, in practice, the glaze may not form a true surface layer and the glazing material will penetrate the thickness of the strip to some extent. Similarly, the subsequently applied labelled reagent may also penetrate the strip. Nevertheless, the essential objective of reducing any interaction between the labelled reagent and the carrier material forming the strip will be achieved. An aqueous sample deposited in receiving member 506 can flow therefrom along the length of strip 510 and in so doing, will dissolve glaze 519 and mobilise the labelled reagent, and carry the labelled reagent along the strip and through zone 517.

Embodiment 4

Figures 11 and 12 illustrate another embodiment of the invention, which is seen in plan view in Figure 11 and in cross-section in Figure 11, the cross-section being an elevation on the line A seen in Figure 11.

Referring to Figure 11, the test device comprises a flat rectangular casing 600 incorporating a centrally disposed rectangular aperture 601, adjacent the left hand end 602, and two further apertures 603 and 604 near the mid point of the device and arranged such that apertures 601, 603 and 604 lie on the central longitudinal axis of the device corresponding to line A. Although all three apertures are illustrated as being rectangular, their actual shape is not critical.

Referring to the cross-section seen in Figure 12, the device is hollow and incorporates within it a porous sample receiving member adjacent end 602 of casing 600 and lying directly beneath aperture 601. A test strip of similar construction to that described with reference to Embodiment 4, comprising a porous strip 606 backed by a transparent plastics sheet 607 is also contained within casing 600, and extends from the porous receiving member 602, with which the porous carrier is in liquid permeable contact, to the extreme other end of the casing. The transparent backing sheet 607 is in firm contact with the upper inner surface 608 of casing 600, and provides a seal against apertures 603 and 604 to prevent ingress of moisture or sample into the casing. Although not shown in the drawings, the porous test strip 606 will incorporate a labelled specific binding reagent, and a test zone and a control zone placed appropriately in relation to apertures 603 and 604, in a manner analagous to that described in Embodiment 3.

In operation, an aqueous sample can be applied through aperture 601, e.g. by means of a syringe, to saturate porous receiving member 605. Thereafter, the aqueous sample can permeate the test strip and after an appropriate time the test result can be observed through apertures 603 and 604.

Embodiment 5

A yet further embodiment of the invention is illustrated in Figures 13 and 14 of the accompanying drawings. Figure 13 shows a device comprising a rectangular casing 700 having in its upper surface 701 a rectangular aperture 702. One end wall 703 of the device 703 incorporates an aperture 704 through which a porous test element communicates with the exterior of the device. Aperture 702 is situated in surface 701 at a point relatively remote from the end 703 containing the aperture 704.

Figure 14 shows a partially cut-away view of the device in Figure 13. The hollow device incorporates a porous test strip 705, running almost the entire length of casing 700 from aperture 704. Test strip 705 incorporates a first zone 706 containing a labelled specific binding reagent and a further zone 707, remote from aperture 704, incorporating an immobilised specific reagent: Zone 706 lies directly beneath aperture 702 is therefore observable from outside casing. Beneath strip 705 and adjacent zone 707, is a crushable element 708 containing one or more substrates or reagents which can be used to produce a detectable signal when released into zone 707, if labelled reagent from 706 has become bound in zone 707 following use of the device. Release of the reagents from member 708 can be effected by applying pressure to the outside of

the casing at that point in order to crush the member and express the reagent therefrom.

In operation, the first test element can be exposed to an aqueous sample, e.g. by dipping end 703 of casing 700 into a vessel containing the sample. The liquid sample will then permeate the length of test strip 705, taking labelled reagent from zone 706 and passing through zone 707 where the labelled reagent can become bound e.g. through a "sandwich" reaction involving an analyte in the sample. When the sample has permeated the test strip, reagent can be released from the crushable member 708 and the result of the test observed through aperture 702.

By way of example only, certain preferred test strip materials, reagents, and methods for their production will now be described.

1. Selection of Liquid Conductive Material

Representative examples of liquid conductive materials include paper, nitrocellulose and nylon membranes. Essential features of the material are its ability to bind protein; speed of liquid conduction; and, if necessary after pre-treatment, its ability to allow the passage of labelled antibodies along the strip. It this is a direct label, it may be desirable for the material to allow flow of particles of size up to a few microns (usually less than 0.5μ). Examples of flow rates obtained with various materials are given below:

	Pore size	Time to Flow 45mm (minutes)
Schleicher + Schuell nitrocellulose (unbacked)	3µ	3.40
	5µ	3.30
	8µ	3.00
	12µ	2.20
polyester-backed	8µ (nominal)	3.40
Whatman Nitrocellulose	5	19.20
Pall "Immunodyne" (nylon)	3	4.00
	5	3.20

The speed of a test procedure will be determined by the flow rate of the material employed and while any of the above materials can be used some will give faster tests than others.

Nitrocellulose had the advantage of requiring no activation and will immobilise proteins strongly by absorbtion. "Immunodyne" is pre-activated and requires no chemical treatment. Papers, such as Whatman 3MM, require chemical activation with for example carbonyldiimidazole in order to successfully immobilise antibody.

2. Labels

Preparation of Labels

A selection of labels which may be used are described below. This list is not exhaustive.

A) Gold Sol Preparation

Gold sols may be prepared for use in immunoassay from commercially-available colloidal gold, and an antibody preparation such as anti-alpha human chorionic gonadotrophin. Metallic sol labels are described, for example, in European patent specification No. EP 7654.

For example, colloidal gold G20 (20nm particle size, supplied by Janssen Life Sciences Products) is adjusted to pH 7 with 0.22µ filtered 0.1M K_2CO_3, and 20mls is added to a clean glass beaker. 200µl of anti-alpha hCG antibody, prepared in 2mM borax buffer pH9 at 1mg/ml, and 0.22µ filtered, is added to the gold sol, and the mixture stirred continuously for two minutes. 0.1M K_2CO_3 is used to adjust the pH of the antibody gold sol mixture to 9, and 2mls of 10% (w/v) BSA is added.

The antibody-gold is purified in a series of three centrifugation steps at 12000g, 30 minutes, and 4°C, with only the loose part of the pellet being resuspended for further use. The final pellet is resuspended in 1% (w/v) BSA in 20mM Tris, 150mM NaCl pH 8.2.

B) Dye Sol Preparation

Dye sols (see, for example, European patent specitication No. EP 32270) may be prepared from commercially-available hydrophobic dyestuffs such as Foron Blue SRP (Sandoz) and Resolin Blue BBLS (Bayer). For example, fifty grammes of dye is dispersed in 1 litre of distilled water by mixing on a magnetic stirrer for 2-3 minutes. Fractionation of the dye dispersion can be performed by an initial centrifugation step at 1500g for 10 minutes at room temperature to remove larger sol

particles as a solid pellet, with the supernatant suspension being retained for further centrifugation.

The suspension is centrifuged at 3000g for 10 minutes at room temperature, the supernatant being discarded and the pellet resuspended in 500mls distilled water. This procedure is repeated a further three times, with the final pellet being resuspended in 100mls distilled water.

The spectra of dye sols prepared as described above can be measured, giving lambda-max values of approximately 657nm for Foron Blue, and 690nm for Resolin Blue. The absorbance at lambda-max, for 1cm path length, is used as an arbitrary measure of the dye sol concentration.

C) Coloured Particles

Latex (polymer) particles for use in immunoassays are available commerically. These can be based on a range of synthetic polymers, such as polystyrene, polyvinyltoluene, polystyrene-acrylic acid and polyacrolein. The monomers used are normally water-insuluble, and are emulsified in aqueous surfactant so that monomer mycelles are formed, which are then induced to polymerise by the addition of initiator to the emulsion. Substantially sperical polymer particles are produced.

Coloured latex particles can be produced either by incorporating a suitable dye, such as anthraquinone, in the emulsion before polymerisation, or by colouring the pre-formed particles. In the latter route, the dye should be dissolved in a water-immiscible solvent, such a chroroform, which is then added to an aqueous suspension of the latex particles. The particles take up the non-aqueous solvent and the dye, and can then be dried.

Preferably such latex particles have a maximum dimension of less than about 0.5 micron.

Coloured latex particles may be sensitised with protein, and in particular antibody, to provide reagents for use in immunoassays. For example, polystyrene beads of about 0.3 micron diameter, (supplied by Polymer Laboratories) may be sensitised with anti-alpha human chorionic gonadotrophin, in the process described below:

0.5ml (12.5mg solids) of suspension is diluted with 1ml of 0.1M borate buffer pH 8.5 in an Eppendorf vial. These particles are washed four times in borate buffer, each wash consisting of centrifugation for 3 minutes at 13000 rpm in an MSE microcentrifuge at room temperature. The final pellet is resuspended in 1ml borate buffer, mixed with 300µg of anti-alpha hCG antibody, and the suspension is rotated end-over-end for 16-20 hours at room temperature. The antibody-latex suspension is centrifuged for 5 minutes at 13000rpm, the supernatant is discarded and the pellet resuspended in 1.5mls borate buffer containing 0.5 milligrammes bovine serum albumin. Following rotation end-over-end for 30 minutes at room temperature, the suspension is washed three times in 5mg/ml BSA in phosphate buffered saline pH7.2, by centrifugation at 13000 rpm for 5 minutes. The pellet is resuspended in 5mg/ml BSA/5% (w/v) glycerol in phosphate buffered saline pH 7.2 and stored at 4°C until used.

(A) <u>Anti-hCG - Dye Sol Preparation</u>

Protein may be coupled to dye sol in a process involving passive adsorption. The protein may, for example, be an antibody preparation such as anti-alpha human chorionic gonadotrophin prepared in phosphate buffered saline pH 7.4 at 2 milligram/ml. A reaction

mixture is prepared which contains 100µl antibody solution, 2mls dye sol, 2mls 0.1M phosphate buffer pH 5.8 and 15.9mls distilled water. After gentle mixing of this solution, the preparation is left for fifteen minutes at room temperature. Excess binding sites may be blocked by the addition of, for example, bovine serum albumin: 4mls of 150mg/ml BSA in 5mM NaCl pH 7.4 is added to the reaction mixture, and after 15 minutes incubation at room temperature, the solution is centrifuged at 3000g for 10. minutes, and the pellet resuspended in 10mls of 0.25% (w/v) dextran/0.5% (w/v) lactose in 0.04M phosphate buffer. This antibody-dye sol conjugate is best stored in a freeze dried form.

(B) LH - Dye Sol Preparation

Due to the structural homology between the alpha subunits of hCG and LH, alpha hCG antibody can be used to detect LH in a cross-reactive immunoassay. Thus, a labelled antibody may be prepared for use in an LH assay in an identical manner to that described in Example 1, using anti-alpha hCG antibody.

3. Preparation of Reagent Strip

Zonal Impregnation of Liquid-conductive Materials

Liquid-conducting material with a restricted zone of immobilised protein, particularly antibody, can be prepared for example as follows:

A rectangular sheet of Schleicher and Schuell backed 8µ nitrocellulose measuring 25cm in length and 20cm in width may have a reaction zone formed upon it by applying a line of material about 1mm wide at 5cm intervals along its length and extending throughout its 20cm width. The

material can, for example, be a suitably selected antibody preparation such as anti-beta (human chorionic gonadotropin) of affinity Ka at 10^9, prepared in phosphate buffered saline pH 7.4 at 2 milligram/ml, suitable for immunoassay of human chorionic gonadotrophin using a second (labelled) anti-hCG antibody in a sandwich format. This solution can be deposited by means of a microprocessor-controlled microsyringe, which delivers precise volumes of reagent through a nozzle, preferably 2mm diameter. When the applied material has been allowed to dry for 1 hour at room temperature, excess binding sites on the nitrocellulose are blocked with an inert compound such as polyvinyl alcohol (1% w/v in 20mM Tris pH 7.4) for 30 minutes at room temperature, and sheets are thoroughly rinsed with distilled water prior to drying for 30 minutes at 30°C.

In one embodiment, the liquid conductive material can then be cut up into numerous strips 5cm in length and 1cm in width, each strip carrying a limited zone of the immobilised antibody to function as an immunosorbent part way (e.g. about half way) along its length. In this example the test strip is used with a liquid label which is mixed with sample. In use, this limited zone then becomes a test reaction zone in which the immunoassay reactions take place.

In another embodiment, the label may be dispensed/ deposited into/on a restricted zone before cutting up the liquid-conductive material into strips. By way of example, this reagent may be dye sol or dye polymer-conjugated anti-hCG antibody prepared as described under dye sol preparation, said reagent being retained in the zone when the material is in the dry state but which is free to migrate through the carrier material when the material is moistened, for example, by the application of

liquid sample containing the analyte to be determined. This mobile reagent zone is applied, for example, as follows:

A sheet of Schleicher and Schuell backed 8μ nitrocellulose, 25cm in length and 20cm in width with zones of immobilised antibody at 5cm intervals along its length, is prepared as described previously. Prior to the deposition of dye labelled antibody, a sublayer of, for example, 60% w/v of sucrose in distilled water is applied by airbrush on the microprocessor controlled system at 6cm intervals along the length of the sheet. Then several passes (e.g. three) of dye labelled antibody prepared in 1% methacel KAM (Trademark for methylcellulose from Dow Chemical Company) and 0.6% (w/v) polyvinylalcohol are applied by airbrush or by microsyringe directly on top of the sublayer. Sheets are then allowed to dry, and cut into strips 5cm in length and 1cm in width, to be used in the completed device.

Gold sols, or coloured polystyrene particles can be deposited by a similar process.

In addition to the test zone various control zone options can be operated. For example a zone of anti-species IgG may be deposited after the test zone.

4. Sandwich Assays Using Strip Format

A sandwich-type reaction may be performed for the detection of human chorionic gonadotrophin (hCG) in a liquid sample. Preferably the label used is a direct label which is readily visible to the naked eye. Dye sols, gold sols or coloured latex particles may be linked to anti hCG antibody, as described above.

With direct labels, assays may be performed in which fresh urine samples are applied directly from the urine stream, or by delivering an appropriate volume (e.g. 100µl) from a container using a pipette to the absorbent wick of the test device. Each sample is allowed to run for five minutes in the device, and the colour generated at the reactive zone read either by eye, or using a light reflectometer.

Indirect labels such as enzymes e.g. alkaline phosphatase may also be used, but require the addition of substrate to generate a coloured endpoint.

Enzyme assays may be performed in which the anti-hCG antibody is conjugated to alkaline phosphatase, using conventional techniques, and diluted 1/100 in 0.01M phosphate buffered saline pH 7 containing 3% polyethylene glycol 6000, 1% (w/v) bovine serum albumin and 0.02% Triton X305 (Trademark - obtainable from Rohm and Haas) before application to the sheet. Fresh urine samples are then applied, either directly from the urine stream, or by delivering an appropriate volume (e.g. 100µl) from a container using a pipette, to the absorbent wick of the test device. Each sample is allowed to run for five minutes before a pad of liquid-swellable material soaked in BCIP substrate (at 1mg/ml in 1M Tris/HCl pH 9.8) is placed in contact with the immobile antibody zone. After a further five minutes, the pad is removed, and colour generated read either by eye, or by using a light reflectometer.

A similar embodiment can be prepared using lutenising hormone (LH) instead of hCG.

5. Competitive Assays

A competitive type assay may be performed as exemplified by estrone-3-glucuronide, a urinary metabolite of estrone. Conjugates of estrone-3-glucuronide and bovine serum albumin are prepared as follows:

Preparation of BSA - Estrone-3-glucuronide

The conjugation of E-3-G and BSA may be achieved through the use of a mixed anhydride. All of the glassware, solvents and reagents employed in the preparation of the activated species must be thoroughly dried using an oven, dessicator or molecular sieves, as appropriate, for at least 24 hours.

Solutions of E-3-G (2nM) in dry dimethylformamide (DMF) and tri-n-butylamine (TnB) (10nM) in dry DMF were equilibrated separately at 4°C. Using pre-cooled glassware E-3-G in DMF (1.25ml) and TnB in DMF (0.25ml) were added to a pre-cooled 5ml Reactivial containing a magnetic stirrer. A solution of isobutyl chloroformate in dry DMF (10nM) was prepared and an aliquot (0.25ml) was cooled to 4°C and added to the Reactivial. The contents of the Reactivial were stirred for 20 minutes at 4°C and a solution of BSA (1mg/ml) in bicarbonate buffer (0.5%) was prepared. When the mixed anhydride incubation was complete, the contents of the Reactivial were added to the BSA solution (2.5ml) and stirred on a magnetic stirrer for 4 hours at 4°C. The conjugate preparation was purified by passage through a Tris buffer equilibrated Pharmacia PD-10 Sephadex G-25 column, transferred to an amber glass storage bottle and stored at 4°C.

Preparation of BSA - E-3-G dye Sol

A dispersion of dye (5% w/v) in distilled water was prepared with thorough mixing and aliquots were centrifuged at 3850rpm (1500g) for 10 minutes in a bench top centrifuge. The pellet was discarded and the supernatant was retained and centrifuged in aliquots at 4850rpm (3000g) for 10 minutes in a bench top centrifuge. The supernatant was discarded and the pellet was resuspended in half of its original volume in distilled water. This step was repeated four times to wash the pellet. The pellet was finally resuspended in distilled water and the absorbance at lambda max was determined.

Solutions of dye sol in distilled water and E-3-G/BSA conjugate diluted in phosphate buffer were mixed to give final concentrations of 10µg/ml conjugate (based on BSA content) and an extrapolated dye sol optical density of 20 at the absorbance maximum. The reaction mixture was incubated for 15 minutes at room temperature and blocked for 15 minutes at room temperature with BSA in a NaCl solution (5mM, pH7.4) to yield a final BSA concentration of 25mg/ml. The reaction mixture was centrifuged at 4850rpm (3000g) for 10 minutes in a benchtop centrifuge, the supernatant was discarded and the pellet was resuspended in half of its original volume in Dextran (0.25% w/v)/Lactose (0.5% w/v) phosphate (0.04M pH5.8) buffer.

Preparation of E-3-G Test Strips

Antibodies to E-3-G were deposited as described in example 3. BSA - E-3-G dye sol was deposited on the strips as described in 3.

Determination of E-3-G

Using reagents described above, a standard curve can be generated by running strips with samples with known concentrations of E-3-G. The colour at the immobile zone can be read, for example using a Minolta chromameter, and the concentration of E-3-G calculated by extrapolating from the reflectance value.

The invention described herein extends to all such modifications and variations as will be apparent to the reader skilled in the art, and also extends to all combinations and subcombinations of the features of this description and the accompanying drawings.

Claims

1. An analytical test device comprising a hollow casing constructed of moisture impervious solid material containing a dry porous carrier which communicates directly or indirectly with the exterior of the casing such that a liquid test sample can be applied to the porous carrier, the device also containing a labelled specific binding reagent for an analyte which labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilised in a detection zone on the carrier material and is therefore not mobile in the moist state, the relative positioning of the labelled reagent and detection zone being such that liquid sample applied to the device can pick up labelled reagent and thereafter permeate into the detection zone, and the device incorporating means enabling the extent (if any) to which the labelled reagent becomes bound in the detection zone to be observed.

2. An analytical test device comprising a hollow casing constructed of moisture- impervious solid material containing a dry porous carrier which communicates directly or indirectly with the exterior of the casing such that a liquid test sample can be applied to the porous carrier, the carrier containing in a first zone a labelled specific binding reagent for an analyte, which labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilised on the carrier material and is therefore not mobile in the moist state, the two zones being arranged such that liquid

sample applied to the porous carrier can permeate via the first zone into the second zone, and the device incorporating means enabling the extent (if any) to which the labelled reagent becomes bound in the second zone to be observed.

3. A test device according to claim 1 or claim 2, wherein the label on the first specific binding reagent is a direct label.

4. A test device according to any one of claims 1 to 3, wherein the casing is constructed of opaque or translucent material, and is provided with at least one aperture through which the analytical result may be observed.

5. A test device according to any one of the preceding claims, within the porous carrier communicates with the exterior of the device via a bibulous sample receiving member which protrudes from the casing and which can act as a reservoir to receive the liquid sample and release it into the porous carrier.

6. A test device according to claim 5, it incorporates a removable and replaceable moisture-impervious cover for the protruding bibulous sample receiving member.

7. A test device according to any one of the preceding claims, within the casing, and cover if present, is moulded from plastics material.

8. A test device according to any one of the preceding claims, wherein the porous carrier comprises a strip or sheet of porous material backed with a layer of transparent moisture-impervious material, the transparent layer being in contact with the inside of the casing

adjacent the aperture(s) to inhibit ingress of moisture or sample.

9. A test device according to claim 7, wherein the porous carrier material is nitrocellulose.

10. A test device according to claim 8, wherein the nitrocellulose has a pore size of greater than about 1 micron.

11. A test device according to any one of the preceding claims, wherein the label comprises coloured latex particles having a maximum dimension of not greater than about 0.5 micron.

12. A test device according to any one of the preceding claims, incorporating a control zone downstream from the second zone in the porous carrier to indicate that the liquid sample has permeated beyond the second zone, the control zone also being observable from outside the casing.

13. A test device according to any one of the preceding claims, wherein the analyte is hCG.

14. A test device according to any one of claims 1 to 12, wherein the analyte is LH.

15. A pregnancy testing device comprising a hollow elongated casing containing a dry porous nitrocellulose carrier which communicates indirectly with the exterior of the casing via a bibulous urine receiving member which protrudes from the casing and which can act as a reservoir from which urine is released into the porous carrier, the carrier containing in a first zone a highly-specific anti-hCG antibody bearing a coloured "direct" label, the

labelled antibody being freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone an highly-specific unlabelled anti-hCG antibody which is permanently immobilised on the carrier material and is therefore not mobile in the moist state, the labelled and unlabelled antibodies having specificities for different hCG epitopes, the two zones being arranged such that a urine sample applied to the porous carrier can permeate via the first zone into the second zone, and the casing being constructed of opaque or translucent material incorporating at least one aperture through which the analytical result may be observed, together with a removable and replaceable cover for the protruding bibulous urine receiving member.

16. A fertile period prediction device, as claimed in claim 15 except that the analyte is LH.

17. A dry porous carrier useful in a device as claimed in claim 1, comprising a strip or sheet of nitrocellulose.

18. A carrier according to claim 17, wherein the nitrocellulose has a pore size of at least about 1 micron.

19. A carrier according to claim 18, wherein the pore size is greater than about 5 microns.

20. A carrier according to claim 19, wherein the pore size is about 8-12 microns.

21. A carrier according to any one of claims 17 to 20 which is backed by a layer of solid water-impervious material.

22. A carrier according to claim 21, wherein the backing material is transparent plastics material.

23. A carrier according to any one of claims 17-22, carrying in a zone thereof an immobilised specific binding reagent for an analyte.

24. A carrier according to claim 23, carrying in another zone thereof a labelled specific binding reagent for the same analyte, which labelled specific binding reagent is freely mobile within the carrier material when in the moist state.

25. A carrier according to claim 24, wherein the label is a direct label.

26. A carrier according to claim 25, wherein the direct label is a coloured latex particle having a maximum dimension of not greater than about 0.5 micron.

27. A carrier according to claim 25 or 26, wherein the zone carrying the labelled reagent has been pre-treated with a glazing material prior to the application of the labelled reagent thereto.

28. A carrier according to claim 27 wherein the glazing material is a sugar.

29. A specific binding assay involving the use of a labelled reagent which is free to migrate through a porous carrier material moistened by the application thereto a an aqueous sample suspected of containing an analyte, wherein the label is a direct label.

30. A specific binding assay according to claim 29, wherein the direct label is a coloured latex particle.

31. A pregnancy test kit suitable for home use, comprising a plurality of devices as claimed in claim 15 individually wrapped in moisture-impervious wrapping and packaged together with appropriate instructions to the user.

32. A fertile period prediction kit suitable for home use, comprising a plurality of devices according to claim 16 individually wrapped in moisture-impervious wrapping and packaged together with appropriate instructions to the user.

33. An analytical test device substantially as hereinbefore described with reference to figures 3 to 5.

34. An analytical test device substantially as hereinbefore described with reference to figures 6 and 7.

35. An analytical test device substantially as hereinbefore described with reference to figures 8 and 9.

36. An analytical test device incorporating a test strip substantially as hereinbefore described with reference to figure 10.

37. An analytical test device substantially as hereinbefore described with reference to figures 11 and 12.

38. An analytical test device substantially as hereinbefore described with reference to figures 13 and 14.

AMENDED CLAIMS
[received by the International Bureau on 23 September 1988 (23.09.88)
original claims 1 and 2 amended; remaining claims unchanged (2 pages)]

1. An analytical test device comprising a hollow casing constructed of moisture impervious solid material containing a dry porous carrier which communicates directly or indirectly with the exterior of the casing such that a liquid test sample can be applied to the porous carrier, the device also containing a labelled specific binding reagent for an analyte or which can participate in a competition reaction in the presence of an analyte, which labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilised in a detection zone on the carrier material and is therefore not mobile in the moist state, the relative positioning of the labelled reagent and detection zone being such that liquid sample applied to the device can pick up labelled reagent and thereafter permeate into the detection zone, and the device incorporating means enabling the extent (if any) to which the labelled reagent becomes bound in the detection zone to be observed.

2. An analytical test device comprising a hollow casing constructed of moisture- impervious solid material containing a dry porous carrier which communicates directly or indirectly with the exterior of the casing such that a liquid test sample can be applied to the porous carrier, the carrier containing in a first zone a labelled specific binding reagent for an analyte or which can participate in a competition reaction in the presence of an analyte, which labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently

immobilised on the carrier material and is therefore not mobile in the moist state, the two zones being arranged such that liquid sample applied to the porous carrier can permeate via the first zone into the second zone, and the device incorporating means enabling the extent (if any) to which the labelled reagent becomes bound in the second zone to be observed.

3. A test device according to claim 1 or claim 2, wherein the label on the first specific binding reagent is a direct label.

4. A test device according to any one of claims 1 to 3, wherein the casing is constructed of opaque or translucent material, and is provided with at least one aperture through which the analytical result may be observed.

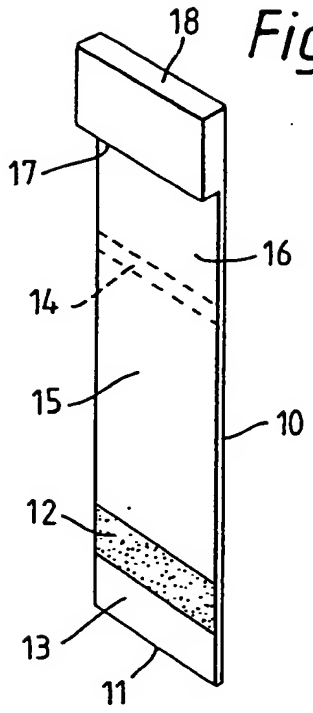
5. A test device according to any one of the preceding claims, within the porous carrier communicates with the exterior of the device via a bibulous sample receiving member which protrudes from the casing and which can act as a reservoir to receive the liquid sample and release it into the porous carrier.

6. A test device according to claim 5, it incorporates a removable and replaceable moisture-impervious cover for the protruding bibulous sample receiving member.

7. A test device according to any one of the preceding claims, within the casing, and cover if present, is moulded from plastics material.

8. A test device according to any one of the preceding claims, wherein the porous carrier comprises a strip or sheet of porous material backed with a layer of transparent moisture-impervious material, the transparent

Fig.1.



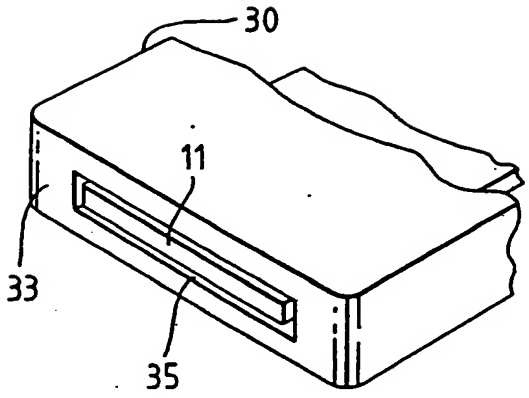
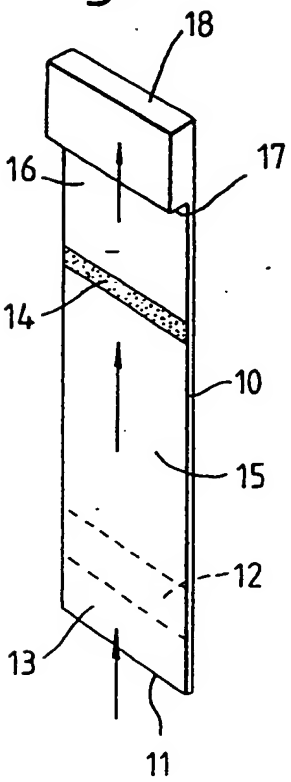


Fig.5.

Fig.2.



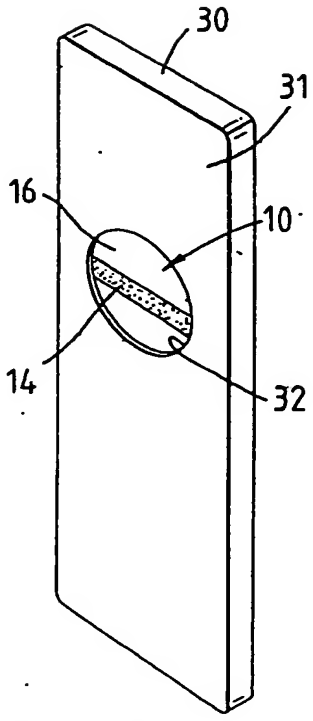


Fig.3.

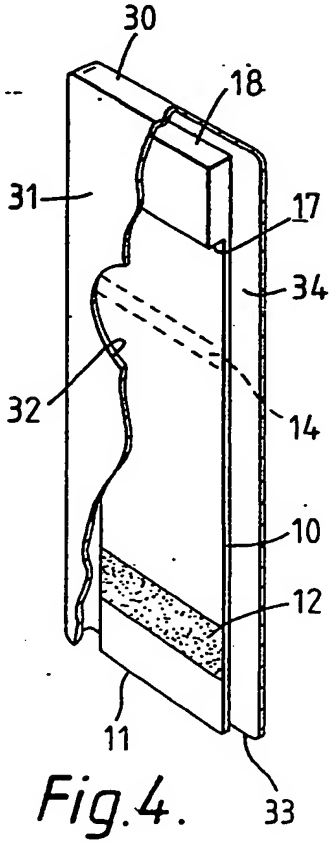
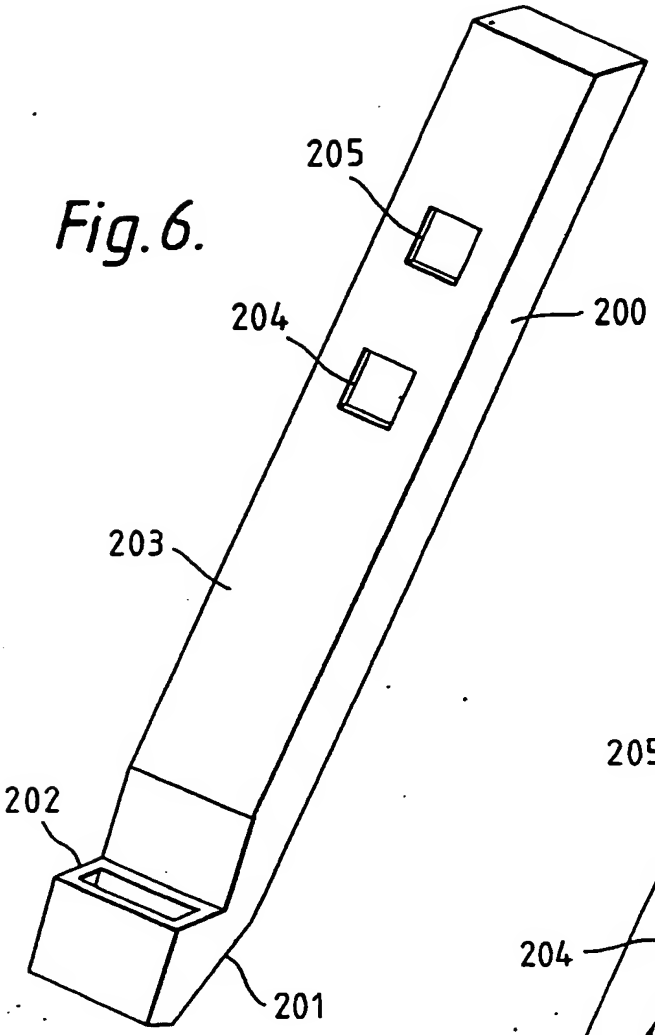


Fig.4.

Fig.6.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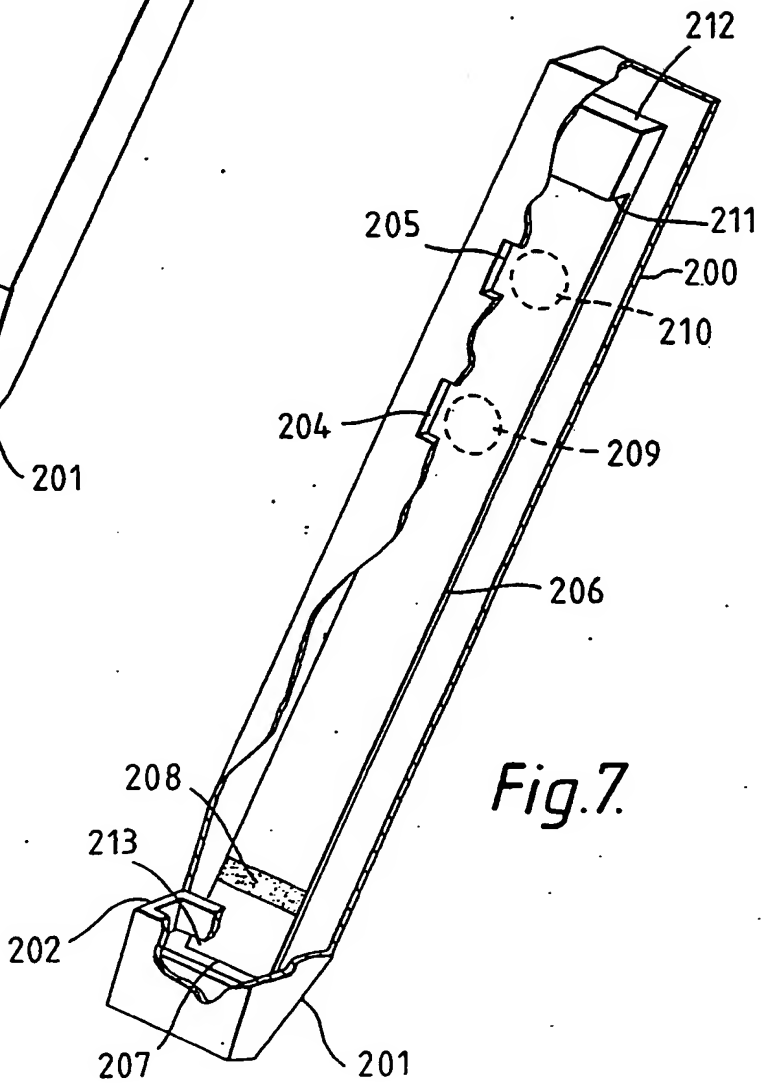
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Fig.7.

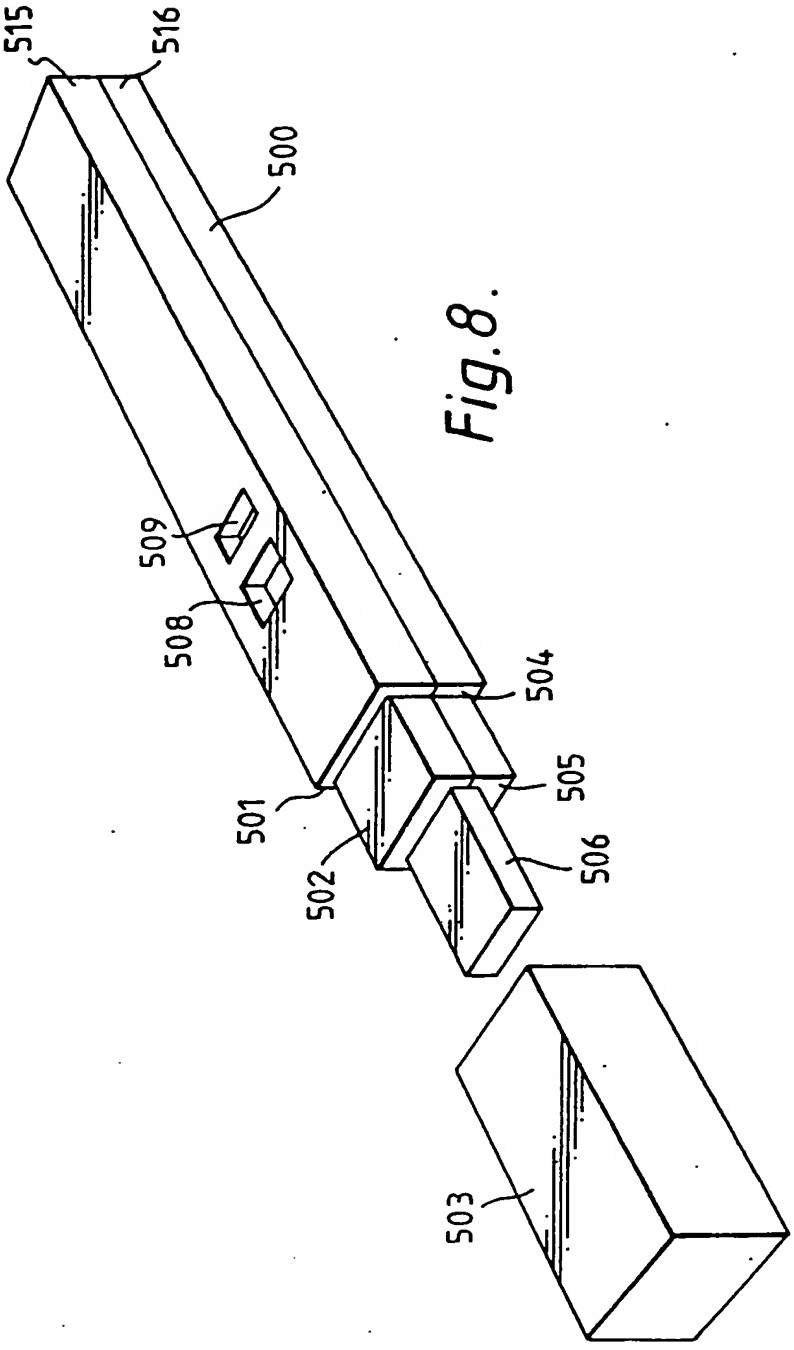
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Fig.8.

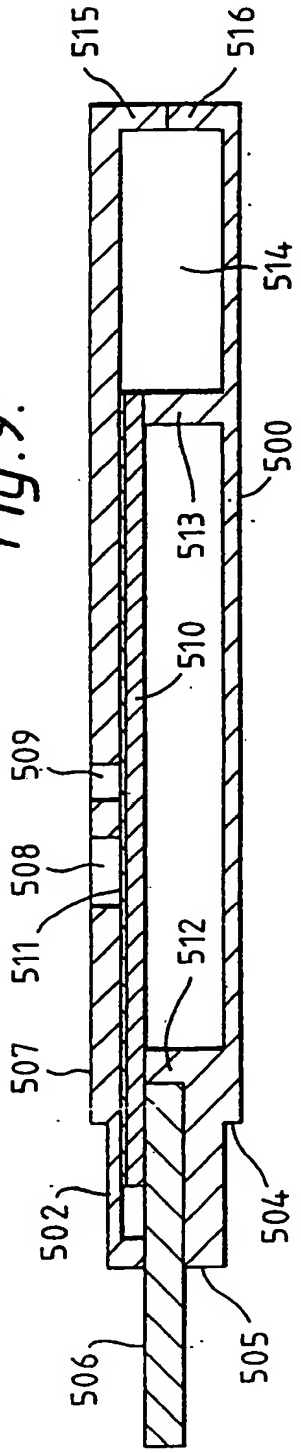
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Fig.9.

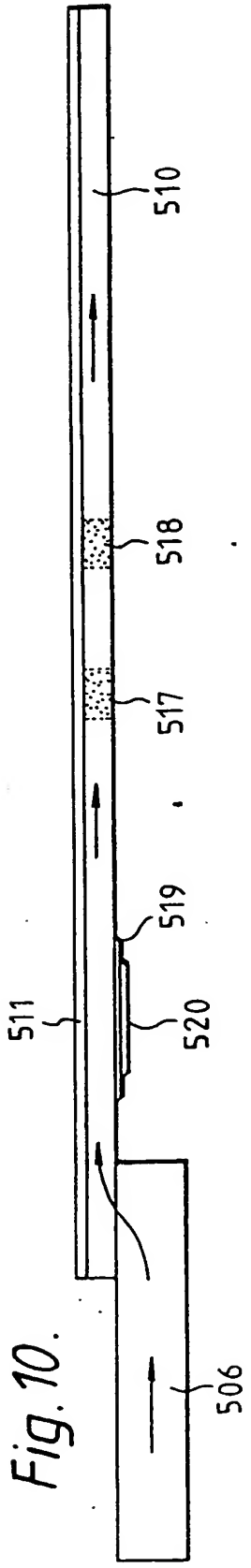

Fig. 10.

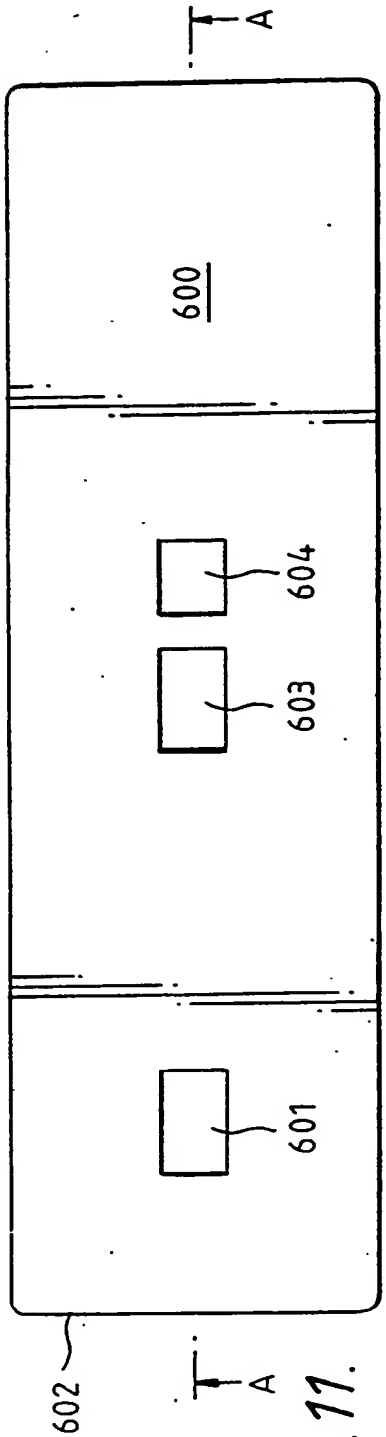

Fig. 11.

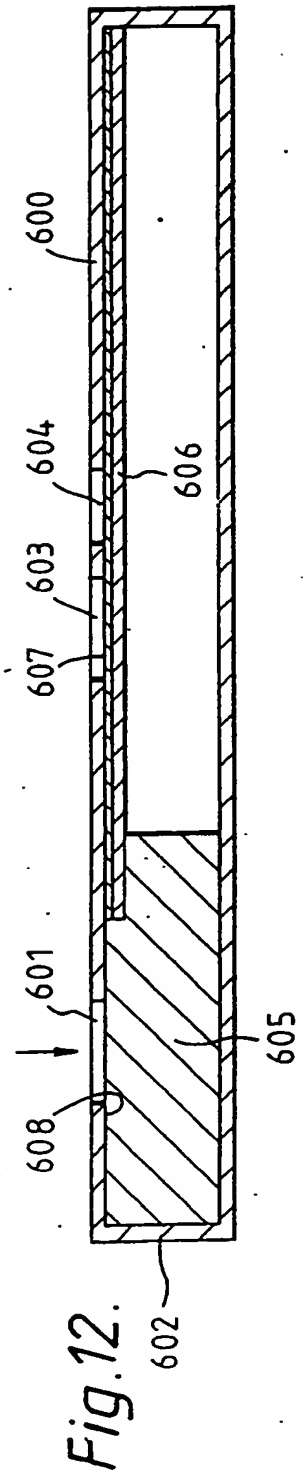

Fig. 12.

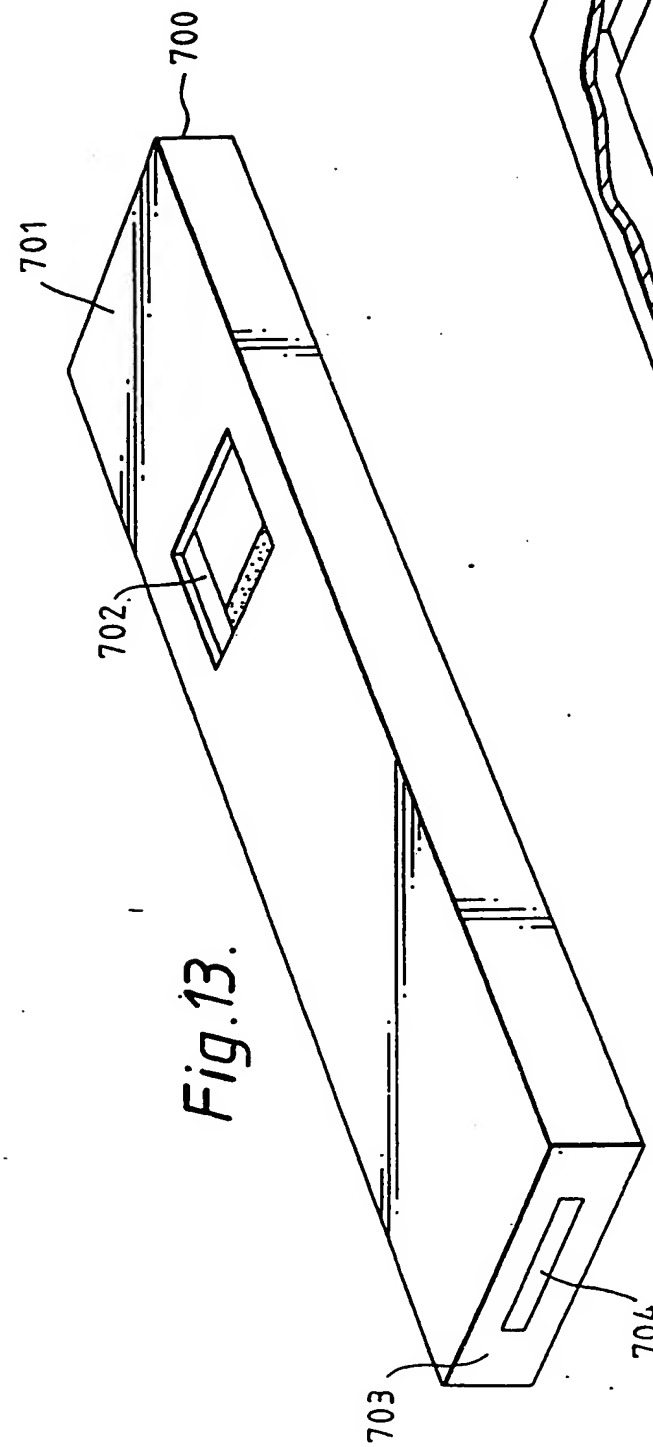

Fig.13.

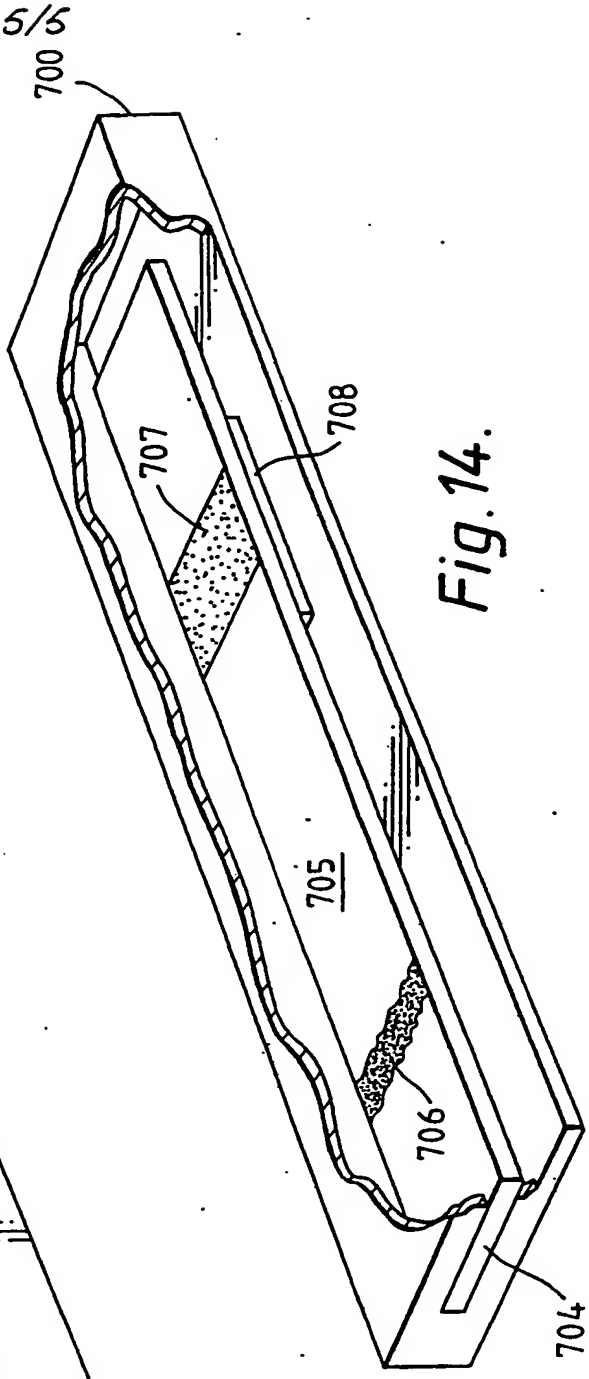

Fig.14.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 88/00322

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: G 01 N 33/543; G 01 N 33/558; G 01 N 33/76; G 01 N 33/74

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X,P	WO, A, 87/02774 (BOOTS-CELLTECH DIAGNOSTICS LTD) 7 May 1987 see the whole document	1,2,4,5, 7,8,12, 33,34
Y		3,9-11,13, 15,17,21-25,29,30
Y	EP, A, 0170746 (COVALENT TECHNOLOGY CORP.) 12 February 1986 see page 17, lines 13-19; claims	3,9-11,13, 15,17,21-25,29,30
Y	EP, A, 0191640 (SYNTEX (U.S.A.) INC.) 20 August 1986 see page 4, line 10 - page 7, line 22; page 11, lines 23-34; page 19, lines 4-27	3,9-11,13, 15,17,21-25,29,30
Y	EP, A, 0149168 (DAIICHI PURE CHEMICALS CO. LTD) 24 July 1985 see the abstract; page 5, line 3 - page 7, line 15 - page 16, line 28; claims; figures	1,2,4,5,7, 14,16

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28th July 1988	30 AUG 1988
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

International Application No. PCT/GB 88/00322

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	EP, A, 0183442 (SYNTEX (U.S.A.) INC.) 4 June 1986 see the abstract; page 4, line 25 - page 5, line 23; figures	1,2,4,5,7, 14,16
A	GB, A, 2016687 (ABBOTT LABS) 26 September 1979 see page 2, lines 24-65	27,28
A	WO, A, 86/04683 (BOEHRINGER BIOCHEMIA ROBIN S.p.A.) 14 August 1986	
A	FR, A, 2356944 (THYROID DIAGNOSTICS, INC.) 27 January 1978 cited in the application	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 8800322 SA 21922

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A- 8702774	07-05-87	EP-A-	0225054	10-06-87
		AU-A-	6592686	19-05-87
		GB-A-	2191578	16-12-87
		JP-T-	63501595	16-06-88
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EP-A- 0191640	20-08-86	JP-A-	61228354	11-10-86
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		US-A-	4740468	26-04-88
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-A- 2356944		SE-B- 443046	10-02-86

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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G01N 33/53, 33/543, 33/544
G01N 33/545, 33/551, 33/566

A1

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(30) Priority data:
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(71) Applicant: ENSYS, INC. [US/US]; 300 Park Offices Drive, Suite 115, Post Office 14063, Research Triangle Park, NC 27709 (US).

(72) Inventor: FRIEDMAN, Stephen, Barry ; 108 Sturbridge Lane, Chapel Hill, NC 27516 (US).

(74) Agents: SIBLEY, Kenneth, D. et al.; Bell, Seltzer, Park & Gibson, P.O. Drawer 34009, Charlotte, NC 28234 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
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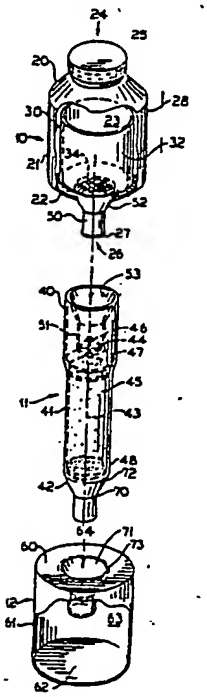
(54) Title: METHOD AND APPARATUS FOR DETECTING ENVIRONMENTAL CONTAMINANTS

(57) Abstract

An apparatus for detecting a target molecule in a liquid sample suspected of containing the same is disclosed. The invention comprises a sample module (10) and a reaction module (11). The sample module (10) has a liquid containing sample chamber (23), a sample chamber inlet opening (24), and a sample chamber outlet opening (26) formed therein. The reaction module (11) has a reaction chamber (43), a reaction chamber inlet opening (44), and a reaction chamber outlet opening (64) formed therein. A ligand which binds the target molecule is immobilized in the reaction chamber (43). A frangible barrier (27) is sealably connected to the sample chamber outlet opening (26) and a pointed member (46) is connected to the reaction chamber inlet (44). The sample module (10) and the reaction module (11) are releasably engaged to one another with the sample chamber outlet opening (26) and the reaction chamber inlet opening (44) in fluid communication.



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METHOD AND APPARATUS FOR DETECTING ENVIRONMENTAL CONTAMINANTS

Field of the Invention

The present invention relates to environmental testing in general, and more particularly relates to methods of environmental testing and modular apparatus for carrying out such tests.

Background of the Invention

Each day toxic chemicals are introduced into the environment through the use of insecticides and herbicides in agriculture, the use of solvents and other chemicals by industry, and from leaking underground storage tanks found at automobile service stations and other facilities. Congress and numerous state governments have responded with legislation requiring the cleanup of contaminated sites. The cost of conducting this cleanup, however, is likely to strain the ability of society to pay for the environmental quality it desires.

The major expense of cleaning up any contaminated site is the cost of treating or disposing of the contaminated material. These costs have increased dramatically in recent years, and similar increases are predicted for the future.

For contaminated soil, the decontamination of which costs two hundred dollars or more per ton, it is prudent to minimize the quantity of clean, uncontaminated soil removed from a site for disposal or treatment. Yet, at the present time there is no fast, easy way to rapidly differentiate contaminated from uncontaminated soil outside the lab and in the field, and no way of providing rapid on-site mapping of contaminated soil in the field.

U.S. Patent No. 4,425,438 to Bauman et al. discloses an assay device comprised of a clear test tube. A capillary tube is suspended in the test tube by means of a funnel, and a cup is fitted to the top of the tube. Glass beads in the capillary tube carry an analytical absorbent such as biotin or avidin. The funnel contains glass beads which carry a primary absorbent. A test substance and an analytical reagent are placed in the cup and permitted to drain through the funnel and into the capillary tube. The order the test substance and the analytical absorbent are placed in the cup is not critical (Column 12, line 67 et seq.). The test substance may be an antigen, the primary absorbent may be an antibody, and the analytical reagent may be a three-member conjugate of the test substance, a detectable group, and a group which binds to the analytical absorbent. The presence of a test substance in a sample displaces the analytical reagent from the primary absorbent into the analytical absorbent, with the amount of analytical reagent present in the analytical absorbent being proportional to the amount of test substance bound to the primary absorbent. Only a limited quantity of test substance can be processed through the device. Moreover, the passing of excess test substance through the device can lead to interference and variability in the test from the sample matrix.

U.S. Patent No. 4,787,971 to Donald concerns a column chromatography separation device for separating chemicals from an eluent fluid. The apparatus includes a tubular column, a receptacle container, a cap member, and an adapter. The tubular column contains an affinity chromatography media. The

device is adapted for laboratory use, and not field testing for environmental contaminants.

U.S. Patent No. 4,092,408 to Litt discloses a heterogenous assay in which antibody is bound to a support, a biological fluid containing both an antigen and labeled antigen contacted to the support, and unbound labeled antigen then detected. U.S. Patent No. 4,590,157 to Chandler et al. discloses an enzyme-linked immunosorbent assay using urease as the enzyme, urea as the indicator, and dibromo-O-cresolsulfonphthalein as the indicator. Neither of these techniques are adapted to field use.

In view of the foregoing, an object of the present invention is to provide a rapid and convenient method for detecting environmental contaminants in a liquid sample, and apparatus for performing this method, which can be carried out in the field with minimum interference and optimum performance, without the necessity of returning the sample to a laboratory.

A second object of the present invention is to provide a test device in which the sensitivity of the device can be increased by simply increasing the quantity of sample liquid which is passed through the device.

A third object of the present invention is to provide a test device in which the liquid sample matrix does not interfere with the detection step, thereby enabling greater sensitivity and decreased variability in the test.

Summary of the Invention

The foregoing and other objects are achieved by the invention disclosed herein. A first aspect of the present invention is an apparatus for detecting a target molecule in a liquid sample suspected of containing the same. The apparatus comprises a sample module and a reaction module. The sample module has a sample chamber, a sample chamber inlet opening, and a sample chamber outlet opening formed therein. Closure means are operatively associated with the sample chamber outlet opening for retaining a liquid sample in the sample chamber. The reaction module has a reaction chamber

and a reaction chamber inlet opening formed therein. Target molecule detection means are contained within the reaction chamber. Means are provided on the sample module and on the reaction module for joining the modules with the sample chamber outlet opening and the reaction chamber inlet opening in fluid communication. Means are operatively associated with the reaction chamber inlet opening for releasing a liquid sample in the sample chamber past the closure means and into the reaction chamber via the sample chamber outlet opening when the modules are joined.

A second aspect of the present invention is a method of detecting a hapten in a liquid sample suspected of containing the same. The method comprises the following steps: (a) providing an immobilized antibody which binds to the hapten; (b) contacting the liquid sample to the immobilized antibody; (c) removing the liquid sample from contact with the immobilized antibody; then (d) contacting a liquid developer solution to the immobilized antibody, the liquid developer solution containing a labeled molecule which binds to the antibody, with the labeled molecule provided in a quantity such that all of the labeled molecule will bind to the antibody if no hapten was present in the sample; and then (e) detecting the presence or absence of free (i.e., unbound) labeled molecule in the developer solution, with the presence of the unbound labeled molecule of the developer solution indicating the presence of the hapten in the liquid sample.

A third aspect of the present invention is a preferred method of detecting a hapten in a liquid sample suspected of containing the same. The method comprises the following steps: (a) providing a reaction module having a reaction chamber and an antibody which binds to the hapten immobilized in the reaction chamber; (b) passing the liquid sample through the reaction module; followed by (c) bringing an indicator support into fluid-flow association with the reaction module; (d) passing a liquid developer solution through the reaction module and onto the indicator support, the developer solution containing a labeled molecule which binds to the antibody,

with the labeled molecule provided in a quantity such that all of the labeled molecule will bind to the antibody if no hapten was present in the sample; and then (e) detecting the presence or absence of labeled molecule on the indicator support, the presence of the labeled molecule on the indicator support indicating the presence of the hapten in the liquid sample.

Brief Description of the Drawings

FIGURE 1 is an exploded perspective view of an apparatus of the present invention, including a sample module, a reaction module, and a collection module;

FIGURE 2 is an enlarged side sectional view of a sample module and reaction module of the present invention, showing the sample module outlet opening and reaction module inlet opening before being joined;

FIGURE 3 is a top sectional view of a reaction module of the present invention, taken along line 3-3 of Figure 2;

FIGURE 4 is an enlarged side sectional view of a sample module and reaction module similar to Figure 2, except showing the sample module outlet opening and reaction module inlet opening after being joined; and

FIGURE 5 is a perspective view of three (3) reaction modules of the present invention suspended over a test strip, with developer solution being passed through the reaction modules.

Detailed Description of the Preferred Embodiment

As shown in Figure 1, a first embodiment of the present invention comprises a sample module 10, a reaction module 11, and a collection module 12. The modules are constructed of any suitable hard, substantially impermeable, non-reactive material, such as polycarbonate. The apparatus is useful for detecting the presence of any target molecule in a liquid sample, but is preferably employed for detecting haptens in a liquid sample. Many common environmental contaminants are aromatic or aliphatic hydrocarbons which are haptens. Exemplary of such haptens are benzene, toluene, xylene, aldicarb, benzo (a) pyrene, and pentachlorophenol.

The sample module 10 comprises a hollow cylinder member having a top portion 20, a body portion 21, and a bottom portion 22. The body portion has a collection chamber 23 formed therein, the top portion has a collection chamber inlet opening 24 formed therein (covered by a cover 25 as illustrated), and the bottom portion has a collection chamber outlet opening 26 formed therein. The inlet opening has a threaded lip to which a threaded cover may be joined. A frangible, fluid impermeable barrier 27 sealably connected to the collection chamber outlet opening 26 serves as a closure means operatively associated with the sample chamber outlet opening for retaining a liquid sample in the sample chamber. The frangible barrier illustrated comprises an impermeable element (such as a metallic foil) secured to the bottom portion of the sample module with a suitable water impermeable adhesive so as to completely overlie the outlet opening.

The sample module has an internal cylinder 30 which serves as means formed in the collection chamber for delivering a measured quantity of liquid through the outlet opening. The circumference of the internal cylinder is less than the circumference of the collection module, so that a space 31 is defined between the internal cylinder side wall 32 and the collection chamber body portion 21. The height of the internal cylinder side wall 32 is less than the height of the collection chamber body portion 21. The internal cylinder is joined to the bottom portion 22 of the sample module, but not to the top portion 20 of the sample module, so that liquid may flow through the inlet opening 24 and into the internal cylinder 30. The volume of the internal cylinder 30 is calibrated to deliver a predetermined amount of the liquid sample being tested through the outlet opening 26 when the internal cylinder is completely filled. Excess liquid placed in the sample module flows over the top 33 of the internal cylinder side wall into the space 31. Alternative means for delivering a predetermined volume of liquid sample from the sample module are to fill the entire volume of the sample module 10 with the liquid sample, (with the chamber volume

being sized as appropriate) or to fill the sample chamber 23 with the liquid sample up to a preset indicia on the side wall 28 of the sample chamber. A porous filter 34 positioned in the internal cylinder serves as filter means for filtering a liquid sample contained in the sample module prior to passing the sample out of the module through the outlet opening.

The reaction module 11 comprises a tubular column member having a top portion 40, a body portion 41, and a bottom portion 42. The body portion 41 has a reaction chamber 43 formed therein, the top portion 40 has a reaction chamber inlet opening 44 formed therein, and the bottom portion has a reaction chamber outlet opening 45 formed therein. The volume of the reaction chamber 43 is preferably not more than the volume of the predetermined liquid sample delivered by the sample module. A pointed member 46 connected to the reaction chamber, disposed within the inlet opening 44 (see Figure 3) and positioned to rupture the sample chamber frangible barrier 27, serves as a means operatively associated with the reaction chamber inlet opening 44 for releasing the liquid sample past the closure means and into the reaction chamber via the sample chamber outlet opening when the sample module and reaction module are joined (see Figures 2 and 4).

Numerous other closure means and means for releasing the liquid sample are available which would perform equally well in the present invention. For example, a one-way valve or a pop-out insert could be used as the closure means, and a blunt probe could be used as the means for releasing the liquid sample. Still other alternate embodiments will be appreciated by those skilled in the art.

A ligand which binds the target molecule is immobilized in the reaction chamber 43, and serves as a target molecule detection means contained within the reaction chamber. The term "ligand," as used herein, means any molecule which binds selectively to a target molecule with the two together forming a specific binding pair. The term "immobilized," as used herein, means merely that the ligand stays within the reaction module. The ligand is preferably immobilized in the reaction

chamber by binding it to a solid support 45, with the solid support in turn itself immobilized in the reaction chamber. Any solid support can be used, exemplary solid supports including agarose, starch, cellulose, chitin, collagen, synthetic polymers of latex or acrylic derivatives, and inorganic materials such as ceramic and porous silica. Optimally, the support is not reactive with the sample compound and should not non-specifically bind. Suitable ligands include, but are not limited to, antibodies (both monoclonal and polyclonal), peptides, polynucleic acids, antitoxins, chelating agents, enzyme inhibitors, receptor agonists, receptor antagonists, transport proteins, avidin and biotin. Antibodies and chelating agents are preferred. Suitable chelating agents include ethylenediaminetetraacetic acid (EDTA), phosphonoacetic acid, pyrophosphate, dibasic orthophosphate, and crown ethers such as dicyclohexano-18-crown-6, cyclodexetrins, and cryptands. The chelating agents are preferably used to detect metal ions in solutions suspected of containing the same. The ligand is bound to the solid support by any suitable binding, including both chemical or physical binding, but is preferably bound to the solid support covalently.

For detecting haptens, the ligand is preferably an antibody. The method of the present invention is advantageously employed with antibodies having affinity constants of about 10^8 liters per mole or less, and is particularly advantageously employed with antibodies having affinity constants of about 10^7 liters per mole or less.

In the illustrated embodiment, the sample chamber contains a particulate solid support 45 (e.g., silica) to which the ligand is bound. Filter screens 46, 47 joined to the interior side wall of the reaction chamber body portion are included at the upper and lower ends of the reaction chamber to secure the solid support within the reaction chamber, and provide means for immobilizing the solid support in the reaction chamber.

Those skilled in the immunoassay art will appreciate numerous other equally suitable ways to detect the target molecule which comprise alternate embodiments of the present invention. For example, the target molecule could combine with other reagents or ligands in the reaction chamber and pass out through the reaction chamber outlet opening for ultimate collection and/or completion of the detection step.

As shown in detail in Figures 2 through 4, the bottom portion of the sample module has a downwardly projecting nipple 50 and the top portion of the reaction module has an upwardly facing bore 51 configured to receive the downwardly projecting nipple. The nipple 50 and bore 51 serve as means formed on the sample module and on the reaction module for joining the modules with the sample chamber outlet opening 26 and the reaction chamber inlet opening 44 in fluid communication. The nipple 50 and bore 51 have tapered portions 52, 53 which mate with one another to assist in connecting the two modules to one another. Preferably, the outer diameter of the nipple and the inner diameter of the bore are sized so that, when joined, the nipple and bore frictionally engage one another. Other shapes and arrangements of elements for joining the modules are also suitable, so long as they provide for fluid communication from the sample chamber to the reaction chamber via the sample chamber outlet opening.

The collection module 12 has a top portion 60, 63, body portion 61, and closed bottom portion 62. The body portion 61 has a collection chamber 63 formed therein and the top portion has a collection chamber inlet opening formed therein. Preferably, the collection chamber volume is at least as great as the reaction chamber volume, and is more preferably at least twice as great as the reaction chamber volume. The bottom portion of the reaction module has a downwardly projecting nipple 70 and the top portion of the collection module has an upwardly facing bore 71 configured to receive the downwardly projecting nipple. The nipple 70 and bore 71 serve as means formed on the reaction module and on the

collection module for joining these modules with the reaction chamber outlet opening and the collection chamber inlet opening in fluid communication. The nipple and bore have tapered portions 72, 73 which mate with one another to assist in connecting the two modules to one another. As previously, the outer diameter of the nipple and the inner diameter of the bore are preferably sized so that, when joined, the nipple and bore frictionally engage one another.

If desired, the reaction module can be provided filled with a liquid to hydrate the solid support. In this case, a removable cap would be connected to the top portion 40 of the reaction module 11. A frangible, fluid impermeable barrier would then be sealably connected to the reaction chamber outlet opening 45, analogous to the barrier 27 sealably connected to the collection chamber outlet opening 26. The collection module 12 could then be provided with a pointed member connected to the collection chamber inlet opening 64 and positioned to rupture the reaction chamber frangible barrier, all analogous to the pointed member 46 of the reaction chamber.

The operation of the apparatus of the preferred embodiment is best illustrated by Figures 2 and 4. A liquid sample to be tested for the presence or absence of a target molecule is first placed in the sample module 10. The sample chamber outlet opening 26 is then joined to the reaction chamber inlet opening 44, whereby the frangible barrier 27 is ruptured by the pointed member 46 and the liquid sample thereby drained from the sample chamber 23 into the reaction chamber 43. The presence or absence of target molecule in the reaction chamber is then determined.

The detection method of the present invention is exemplified by Figure 5. In the illustrated embodiment, a liquid sample suspected of containing the hapten is first passed through a reaction module. The reaction chamber of the reaction module 11 contains an antibody which binds the hapten. The reaction module 11 is then suspended on a rack 75 over an indicator strip 76 and a liquid developer solution

77 passed through the reaction module onto the test strip. The developer solution contains a labeled molecule which binds to the antibody immobilized in the reaction chamber. The developer solution is provided in a quantity such that all of the labeled molecule will bind to the antibody if no hapten was present in the sample. Thus, if no hapten was present in the liquid sample, then the labeled molecule will not pass through the reaction chamber and onto the test strip. However, if hapten was in fact present in the liquid sample, then at least some of the labeled molecule will pass through the reaction chamber and onto the test strip.

In an embodiment of the foregoing method, the proportion of antibody to labeled molecule in the liquid developer solution is increased by a predetermined amount so that, if hapten is present in the liquid sample but the quantity of the hapten present is below a predetermined level, then all of the labeled molecule will bind to the antibody, none of the labeled molecule will be free, and no indication of the presence of the hapten in the liquid sample will be given. Thus, for example, if one is only concerned with detecting benzene in a sample at concentrations of five parts per billion or more, then the proportion of antibody to labeled molecule is increased so that, for a given quantity of sample liquid, all of the labeled molecule is bound to the antibody even though some antibody is occupied by the benzene. If the benzene concentration exceeds five parts per billion, then the quantity of labeled molecule is such that the antibody binding capacity is exceeded and some of the labeled molecule cannot bind antibody and will remain free and an indication of the presence of benzene in the liquid sample will be given when, for example, the labeled molecule contacts the indicator strip.

The labeled molecule in the developer solution preferably comprises a hapten and a detectable or signal producing group bound to the hapten. The hapten of the labeled molecule may be the same as the hapten for which detection is sought. The detectable group may be any

conventional detectable group, such as an enzyme label, a radioisotope label, a fluorescent label, or a dye polymer. In the preferred embodiment, the detectable group comprises an enzyme, the readout strip comprises a paper strip with an enzyme substrate of the enzyme impregnating the paper strip, and the enzyme and enzyme substrate are selected so that the reaction product of the enzyme and substrate is one which provides a positive color change on the readout strip (the color of the paper strip itself is likewise selected to achieve this result, with the paper strip preferably being a white paper strip). Thus, a color change on the indicator support indicates that the target molecule was present in the liquid sample, and the absence of a color change on the indicator support indicates that the target molecule was not present in the liquid sample. A preferred labeled hapten is a benzene-beta-galactosidase conjugate, and a preferred enzyme substrate is orthonitrophenyl-beta-D-galactopyranoside (ONPG). Beta-galactosidase converts colorless ONPG into a colored ortho-nitrophenyl product. A variety of other substrates are also available which provide the capability of producing different colored reactions (e.g., X-gal., CPRG) or fluorescent reactions (e.g., methyl-umbeliferone-β-D-galactopyranoside (MUG)). An alternate labeled hapten is a benzene-horseradish peroxidase (HRP) conjugate. In this case, the developer contains a chromophore (e.g., orthophenylene diamine) and the readout strip could be paper impregnated with a peroxidase substrate (e.g., hydrogen peroxide, urea peroxide). In the presence of HRP, the peroxidase substrate donates an electron to the chromophore, which then changes color.

Numerous variations can be made on the foregoing. Alternate indicator supports can be used, including particles, beads, and solid cards. The indicator support can be brought into fluid-flow association with the reaction chamber by any technique which permits movement of the liquid developer solution from the reaction chamber to the indicator support. Preferably, fluid-flow association is established by bringing

the indicator support to a position which permits the gravity drainage of the developer solution from the reaction chamber onto the solid support. Alternatively, however, the developer solution could be moved from the reaction chamber onto an appropriately oriented indicator support by centrifugal force, or the indicator support could be immersed in developer solution in the reaction chamber.

The present invention is particularly advantageous for detecting haptens in soil and water samples. When a liquid leachate sample is taken, it will be contaminated by particulate organic and inorganic matter, and the pH may vary from sample to sample. The present invention, by separating the liquid sample matrix from the ligand in the manner described prior to introducing the developer, reduces interference of the antibody and developer conjugate in the binding assay.

Binding events have, for clarity, been described in absolute terms herein (i.e., "all bound," "none bound"). In practice, it will be appreciated that minor deviations from the absolute will occur without altering or detracting from the operation of the present invention.

The foregoing is illustrative of the present invention, and is not to be taken as restrictive thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

THAT WHICH IS CLAIMED IS:

1. An apparatus for detecting a target molecule in a liquid sample suspected of containing the same, comprising:

(a) a sample module having a sample chamber, a sample chamber inlet opening, and a sample chamber outlet opening formed therein;

(b) closure means operatively associated with said sample chamber outlet opening for retaining a liquid sample in said sample chamber;

(c) a reaction module having a reaction chamber and a reaction chamber inlet opening formed therein;

(d) target molecule detection means contained within said reaction chamber;

(e) means formed on said sample module and on said reaction module for joining said modules with said sample chamber outlet opening and said reaction chamber inlet opening in fluid communication; and

(f) means operatively associated with said reaction chamber inlet opening for releasing said liquid sample past said closure means and into said reaction chamber via said outlet opening.

2. An apparatus as claimed in Claim 1, further comprising means formed in said sample chamber for delivering a measured quantity of said liquid sample to said reaction chamber.

3. An apparatus as claimed in Claim 1, wherein said closure means comprises a frangible barrier sealably connected to said sample chamber outlet opening, and wherein said means operatively associated with said reaction chamber inlet opening for releasing said liquid sample comprises a pointed member connected to said reaction chamber, said pointed member disposed within said reaction chamber inlet opening and positioned to rupture said frangible barrier when said modules are joined.

4. An apparatus as claimed in Claim 1, further comprising a collection module having a collection chamber and a collection chamber inlet opening formed therein, and means operatively associated with said reaction chamber outlet opening and with said collection chamber inlet opening for releasably engaging said chambers to one another.

5. An apparatus as claimed in Claim 1, wherein the volume of said sample chamber is greater than the volume of said reaction chamber.

6. An apparatus as claimed in Claim 1, wherein said reaction module comprises a tubular column member having a top portion, a body portion, and a bottom portion, with said reaction chamber inlet opening formed in said top portion, said reaction chamber formed in said body portion, and said reaction chamber outlet opening formed in said bottom portion, and with said reaction chamber inlet opening and said reaction chamber outlet opening axially aligned with said elongate tubular column member.

7. An apparatus as claimed in Claim 1, wherein said target molecule detection means comprises a solid support immobilized within said reaction chamber and a ligand which binds said target molecule bound to said solid support.

8. An apparatus as claimed in Claim 7, wherein said ligand comprises an antibody.

9. An apparatus as claimed in Claim 8, wherein said antibody comprises an anti-hapten antibody.

10. A sample module useful in an apparatus for detecting a target molecule in a liquid sample suspected of containing the same, said sample module comprising:

(a) a hollow cylinder member having a top portion, a body portion, and a bottom portion, with said body portion having a collection chamber formed therein, said top portion having a collection chamber inlet opening formed therein, and said bottom portion having a collection chamber outlet opening formed therein; and

(b) closure means operatively associated with said sample chamber outlet opening for retaining a liquid sample in said sample chamber.

11. A sample module as claimed in Claim 10, further comprising means formed in said collection chamber for delivering a measured quantity of liquid through said outlet opening.

12. A sample module as claimed in Claim 10, further comprising filter means contained within said collection chamber for filtering said liquid sample before said liquid sample passes through said outlet opening.

13. A sample module as claimed in Claim 10, wherein said closure means comprises a frangible barrier sealably connected to said sample chamber outlet opening.

14. A reaction module useful in an apparatus for detecting a small molecule in a liquid sample suspected of containing the same, said reaction module adapted for use with a sample module having a sample chamber, a sample chamber inlet opening, and a sample chamber outlet opening formed therein, said sample module having closure means operatively associated with said sample chamber outlet opening for retaining a liquid sample in said sample chamber, said reaction module comprising:

(a) a tubular column member having a top portion, a body portion, and a bottom portion, with said body portion having a reaction chamber formed therein, said top portion having a reaction chamber inlet opening formed therein, and said bottom portion having a reaction chamber outlet opening formed therein;

(b) target molecule detection means contained within said reaction chamber; and

(c) means operatively associated with said reaction chamber inlet opening for releasing said liquid sample past said closure means and into said reaction chamber via said sample chamber outlet opening when said sample module and said reaction module are joined.

15. A reaction module as claimed in Claim 14, wherein said closure means comprises a frangible barrier sealably connected to said sample chamber outlet opening, and wherein said means operatively associated with said reaction chamber inlet opening comprises a pointed member connected to said reaction chamber, said pointed member disposed within said opening and positioned to rupture said frangible barrier.

16. a reaction module as claimed in Claim 14, wherein said target molecule detection means comprises a solid support immobilized within said reaction chamber and a ligand which binds said target molecule bound to said solid support.

17. A reaction module as claimed in Claim 16, wherein said ligand comprises and antibody.

18. A reaction module as claimed in Claim 17, wherein said antibody comprises an anti-hapten antibody.

19. A reaction module as claimed in Claim 18, wherein said anti-hapten antibody binds to a hapten selected from the class consisting of consisting of benzene, toluene, xylene, aldicarb, benzo (a) pyrene, and pentachlorophenol.

20. A method of detecting a hapten in a liquid sample suspected of containing the same, said method comprising the steps of

(a) providing an immobilized antibody which binds to said hapten;

(b) contacting said liquid sample to said immobilized antibody;

(c) removing said liquid sample from contact with said immobilized antibody; then

(d) contacting a liquid developer solution to said immobilized antibody, said liquid developer solution containing a labeled molecule which binds to said antibody, with said labeled molecule provided in a quantity such that all of said labeled molecule will bind to said antibody if no hapten was present in said sample, and then

(d) detecting the presence or absence of unbound labeled molecule in the developer solution, the presence of unbound labeled molecule in the developer solution indicating the presence of said hapten in said liquid sample.

21. A method according to Claim 20, wherein said labeled molecule is provided in a quantity so that, if not more than a predetermined amount of hapten is present in said liquid sample, then all of said labeled molecule will bind to said immobilized antibody and no hapten will be indicated as present in said solution.

22. A method according to Claim 20, wherein said labeled molecule comprises a molecule which selectively binds to said ligand and a detectable group bound thereto, said detectable group selected from the class consisting of enzyme labels, fluorescent labels, and radioisotope labels.

23. A method according to Claim 20, wherein said detectable group comprises an enzyme label, and wherein said indicator support carries an enzyme substrate, said enzyme label and said enzyme substrate selected so that the reaction product thereof provides a positive color change on said indicator support.

24. A method according to Claim 20, wherein said hapten is selected from the class consisting of benzene, toluene, xylene, aldicarb, benzo (a) pyrene, and pentachlorophenol.

25. A method of detecting a target molecule in a liquid sample suspected of containing the same, said method comprising the steps of:

(a) providing a reaction module having a reaction chamber and a ligand which binds to said target molecule immobilized in said reaction chamber;

(b) passing said liquid sample through said reaction module; followed by

(c) bringing an indicator support into fluid-flow association with said reaction module;

(d) passing a liquid developer solution through said reaction module and onto said indicator support, said developer solution containing a labeled molecule which binds to said ligand, said labeled molecule provided in a quantity such that all of said labeled molecule will bind to said ligand if no target molecule was present in said sample; and then

(e) detecting the presence or absence of labeled molecule on said indicator support, the presence of said labeled molecule on said indicator support indicating the presence of target molecule in said liquid sample.

26. A method according to Claim 25, wherein said labeled molecule is provided in a quantity so that, if not more than a predetermined amount of hapten is present in said liquid sample, then all of said labeled molecule will bind to said immobilized antibody and no hapten will be indicated as present in said solution.

27. A method according to Claim 25, wherein said labeled molecule comprises a molecule which selectively binds to said ligand and a detectable group bound thereto, said detectable group selected from the class consisting of enzyme labels, fluorescent labels, radioisotope labels, and dye polymers.

28. A method according to Claim 25, wherein said detectable group comprises an enzyme label, and wherein said indicator support carries an enzyme substrate, said enzyme label and said enzyme substrate selected so that the reaction product thereof provides a positive color change on said indicator support.

29. A method according to Claim 25, wherein said hapten is selected from the class consisting of benzene, toluene, xylene, aldicarb, benzo(a)pyrene, and pentachlorophenol.

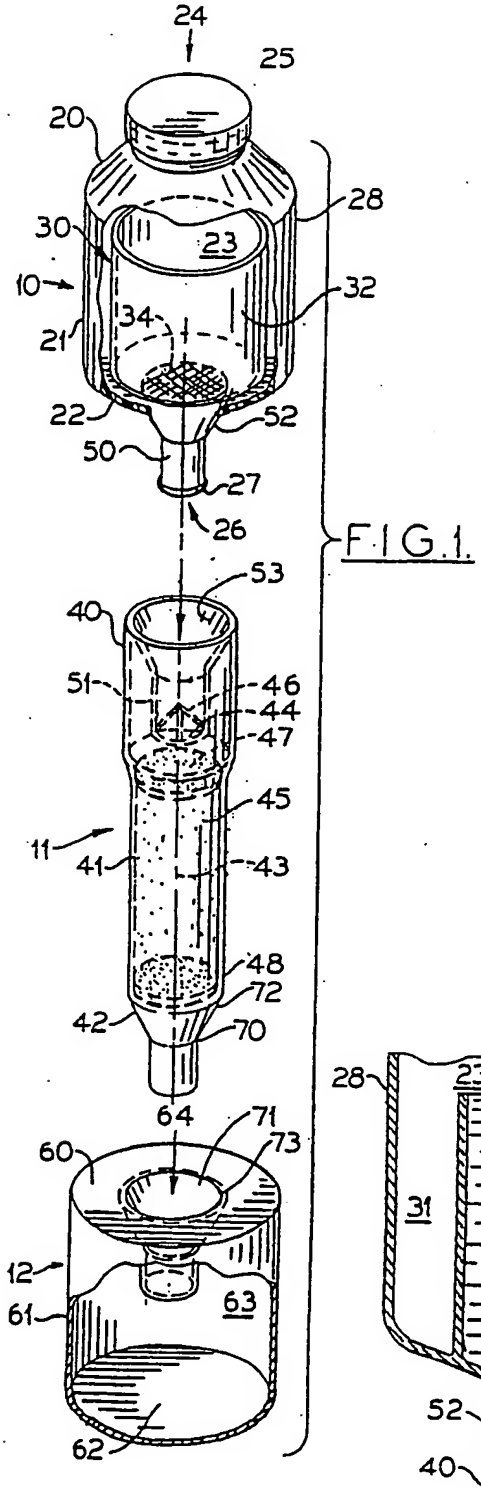

FIG.1.

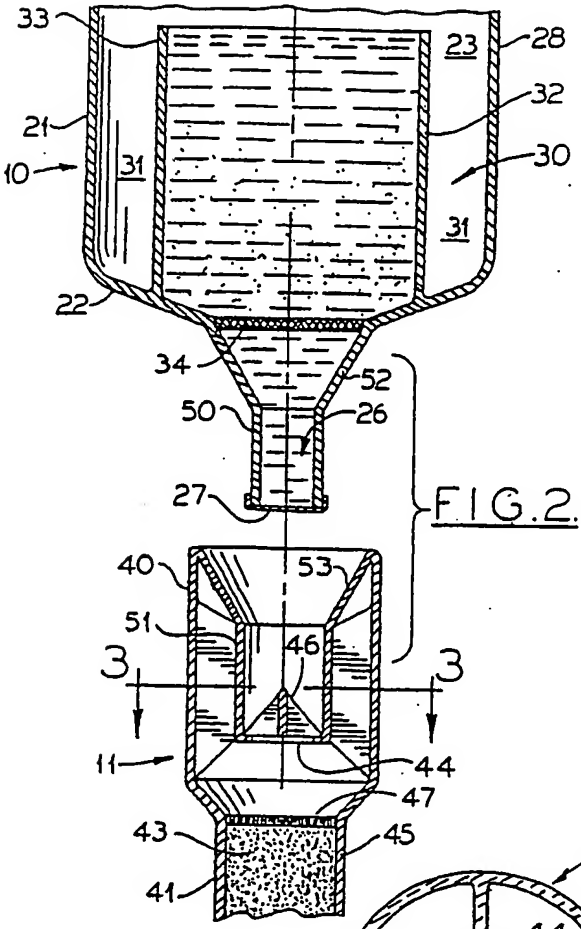

FIG.2.

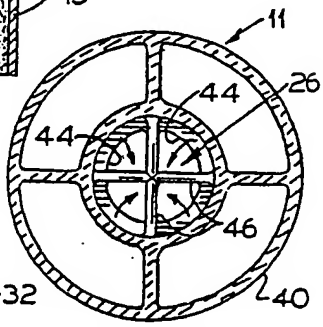

FIG.3.

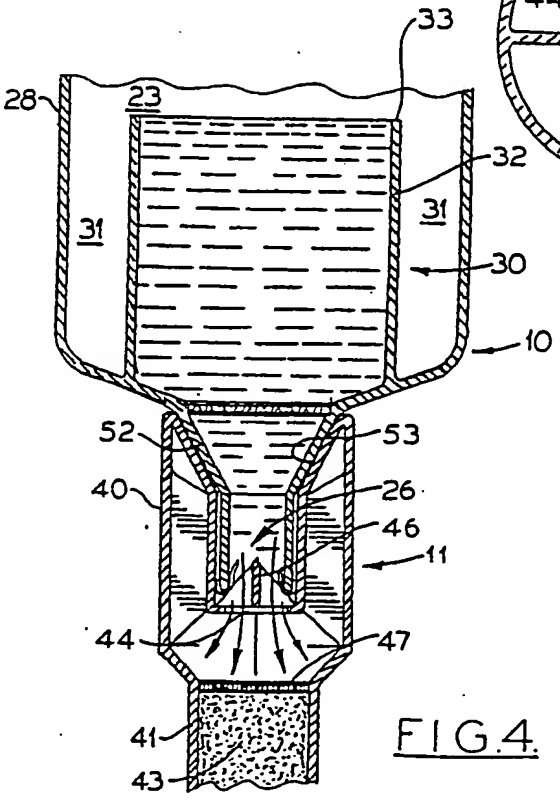

FIG.4.

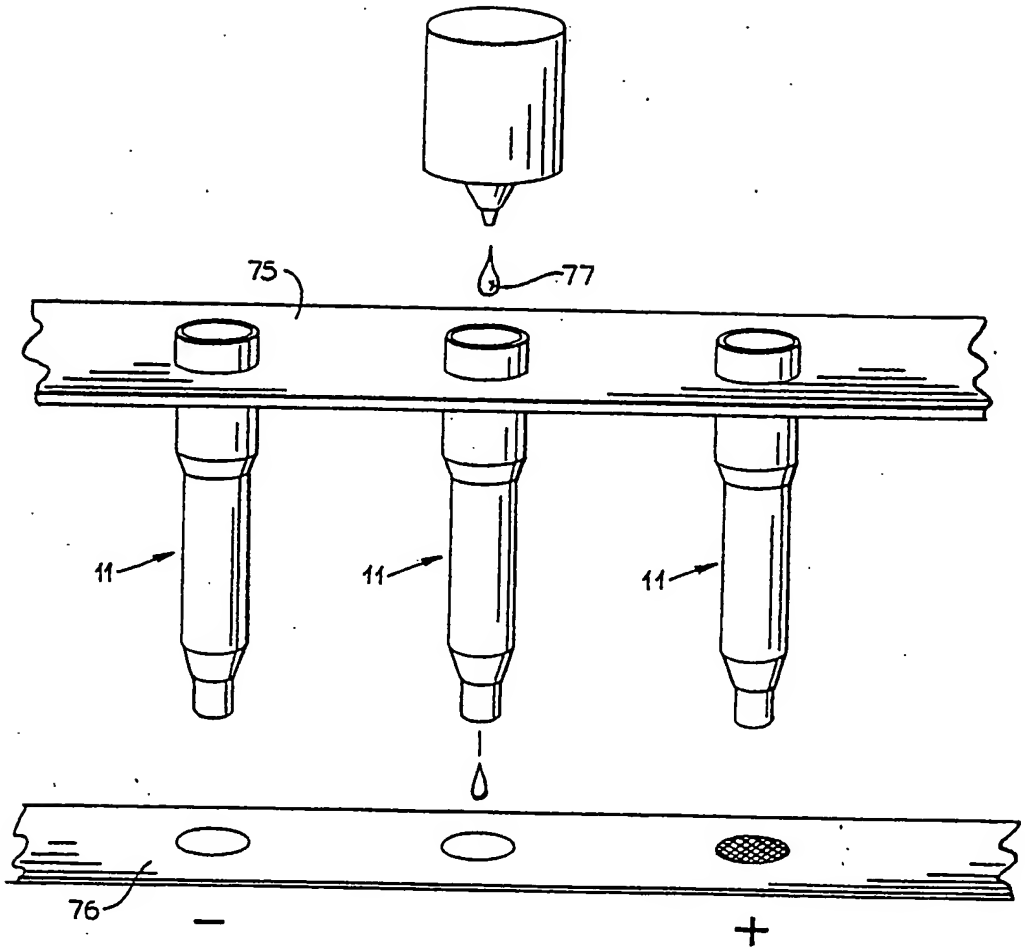

FIG.5.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/02817

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 3

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): B01L 11/00; C12Q 1/64; G01N 33/53, 33/543, 33/544. 33/545, 33/551
U.S.Cl.: 422/58,59,61,69,101; 435/7,9,299,311; 436/501,518,528,531 33/566

II. FIELDS SEARCHED

Minimum Documentation Searched 4

Classification System	Classification Symbols
U S	422/58,59,61,69,101 435/7,9,810,299,311 436/501,518,524,528,531,807,810

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 5

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
X 1	US, A, 4,476,093 (WATANABE et al.) 09 October 1984 See Figure 1; Abstract; column 2, lines 5-11.	14, 16, 17
X	US, A, 3,640,267 (HURTIG et al.) 08 February 1972 See Figure 1; column 1, lines 61-66, 72-75; column 3, lines 5-16.	10, 11, 13
X Y	US, A, 4,608,231 (WITTY et al) 26 August 1986 See abstract; column 2, lines 23-34. Column 5, line 43 - column 6, line 7.	20, 22 21, 23, 25-28
X Y	US, A, 4,208,187 (GIVNER) 17 Jne 1980 See Figures 7, 7A; column 11, lines 23-65; column 12 lines 10-13; column 12, line 59 - column 13, line 47.	10, 11, 13 1-3, 5, 7, 8, 12, 14-17, 20, 22-23, 25, 27-28
Y	US, A, 4,665,034 (CHANDLER) 12 May 1987 See Figure 3; column 1, lines 21-22, 48-52; column 2, lines 51-66; claims 1, 2, 4, 5, 9, 11.	1-27
Y	US, A, 3,888,629 (BAGSHAWE) 10 June 1975 See Figure 1; column 2, line 13 - column 3, line 55; column 4, lines 11-38.	1-27

* Special categories of cited documents: 15

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 2	Date of Mailing of this International Search Report 2
21 August 1990	26 SEP 1990
International Searching Authority 1	**Signature of Authorized Officer 20**
ISA/US	Carol A. Spiegel

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	US, A, 4,632,901 (VALKIRS et al.) 30 December 1986 See column 2; lines 32-60.	20, 22, 23
Y	US, A, 4,775,629 (KUHL et al.) 04 October 1988 See Figures 1-3; column 2, lines 29-63; column 3, line 1 - column 4, line 28.	1, 6, 14, 15
Y	US, A, 4,787,971 (DONALD) 29 November 1988 See Figure 7; column 3, lines 34-43; column 4, lines 52-60; column 5, lines 8-26; column 6, lines 3-24.	1, 6-9, 14-19
A	US, A, 4,324,758 (EISENTRAUT et al.) 13 April 1982 see entire document.	1-29

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . . . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..... , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

I. Claims 1-19 and 25-29 drawn to a first specific binding method of detecting a target molecule and an apparatus designed for said method classified in CLass 435 subclass 7.

II. Claims 20-24 drawn to a second specific binding method for detecting a hapten classified in Class 436 subclass 518.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application. TELEPHONE PRACTICE

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
Y	US, A, 4,734,262 (BAGSHAWE) 29 March 1988 See Figure 1-4; column 1, lines 7-11, 55-65; column 2, lines 24-28; column 2, line 54 - column 3, line 49.	1-9, 14-19, 25-29
Y	US, A, 4,778,751 (EL SHAMI et al.) 18 October 1988 See abstract; column 6, lines 20-64; column 21, line 1 - column 22, line 5.	7-9, 16-29

CONTINUATION SHEET

ATTACHMENT TO PCT/ISA/TELEPHONE PRACTICE

The above inventions lack unity under PCT Rule 13 since the second method of group II requires an immobilized antibody (see claim 20) whereas, the first method and apparatus of group II do not (see claims 1 and 25); e.g. a liquid phase comprising excess labeled molecules and labeled molecules complexed with target molecule might be separated by filtration and the amount of complexed label on, but not bound to, filter be detected. Alternately, the second method of group II does not require the sample module, closure means, fluid communication means or shunting means of the apparatus of group I.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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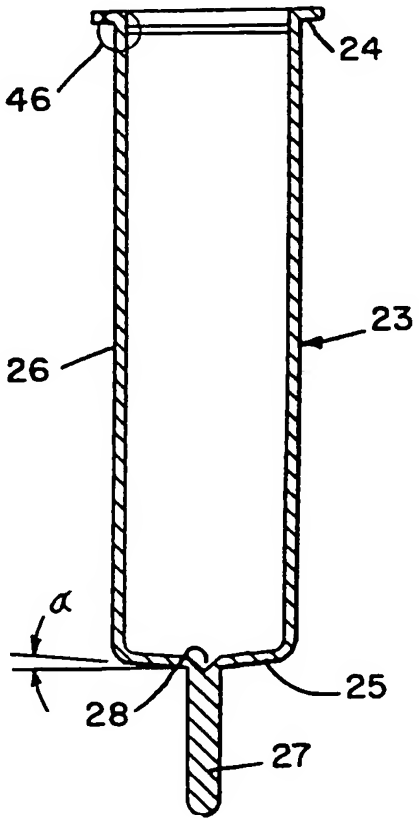
(54) Title: ORAL COLLECTION FOR IMMUNOASSAY

(57) Abstract

A method and device for collecting immunoglobulins and other analytes from the oral cavity for immunological and other testing. The device is a treated absorbent pad used to collect a specimen having a high concentration of immunoglobulins or other analytes. The specimen can be subjected to a basic immunological testing technique which can be used as a tool for screening a patient for diseases. A test kit is also provided.



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ORAL COLLECTION FOR IMMUNOASSAY

TECHNICAL FIELD

The present invention relates to the field of immunological testing. In particular, a system for analyzing immunoglobulins and other substances extracted from the oral cavity is disclosed.

The immune system of the mouth not only interacts with the general immune system of the body, but also has its own centralized center for antigen-antibody response. Within the oral cavity is found lymph nodes and intraoral lymphoid aggregations. The extraoral lymph nodes are involved in the drainage of the oral mucosa, gum and teeth. However, the function of the intraoral lymphoid tissue is little understood.

The extraoral lymph nodes include a fine network of lymph capillaries which are superficially located in the mouth, palate, cheeks, lips, gingiva, and pulp of the teeth. The capillaries join to larger lymph vessels which originate from a network deep in the muscle of the tongue and other structures. An antigen can gain entry into the oral lymphatic system directly through the capillaries or be transported there by phagocytes. Once inside the network, the antigen can induce an immune response.

Included in the intraoral lymphoid tissue are generally four distinct tissue aggregations: (a) the tonsils, (b) scattered submucosal lymphoid aggregations, (c) salivary gland lymphoid tissue, and (d) gingival lymphoid tissue.

The tonsils (palatine and lingual) primarily produce B-cells and T-cells which are generally contained within a cap of lymphocytes and plasma cells. Antigen typically gains entry into the tonsils through a distinct epithelial region wherein the antigen can come into contact with the T- and B-cells to stimulate an immune response. The predominant type of antibody formed in the tonsils is found to be IgG followed, in order, by IgA, IgM, IgD and IgE.

Scattered submucosal lymphoid cells have not been extensively studied. These cell masses are histologically similar to tonsillar tissue.

Both the major salivary glands (parotid, submandibular and sublingual) and the minor salivary glands have been found to contain lymphocytes and plasma cells. Most of the plasma cells secrete IgA and some IgG or IgM. The IgA synthesized in the salivary glands has a dimeric structure. This type of IgA is referred to as secretory IgA (sIgA) and is the major immunoglobulin component in saliva.

Both T-cells and B-cells are found in the gingival lymphoid tissue. In subjects having clinically normal gingival tissue, T-cells predominate. During an infectionary period, such as during the development of gingivitis, B-cells have been found to predominate.

Plasma cells are also found in the gingival lymphoid tissue. Clusters of these cells are generally located near the blood vessels and predominantly produce IgG. To a lesser extent, IgA and IgM are also manufactured. More importantly, Brandtzaeg et al. in, Human Saliva: Clinical Chemistry and Microbiology edited by Jorma O. Tenovuo, have shown that the immunoglobulins from the secretions from the gingival tissue area are directly related to the immunoglobulins found in the blood.

Because of the association between immunoglobulins of the blood and saliva, as well as the occurrence of sIgA peculiar to salival fluid, antigen-antibody tests have been conducted on the saliva to assess the value of such tests as a screening tool for diseases.

Collection of saliva from the salivary glands is complicated by the low volumes secreted, the diverse anatomic dispersion of the glands, and the relatively high viscosity of the fluid. Most techniques for collection involve the use

of capillary tubes, suction into micropipettes, chewing on paraffin or aspiration into polypropylene syringes. These methods, however, are limited in that viscosity of the saliva makes the recovery of bubble-free material by these techniques difficult. Other methods of collection have been suggested to eliminate or at least reduce the quantity of bubbles in the sample. Among such methods include collecting saliva in the mouth by direct absorption with a sponge or flexible wad of osmotic membrane. After absorption, the saliva can be separated from the absorptive material by centrifugation or by compressing the absorptive material. However, absorption is generally accomplished by using cotton, nylon, or polyester as the absorptive material. These materials can non-specifically bind proteins which can result in an undesirably low recovery of immunoglobulins.

BACKGROUND ART

Testing of salivary specimens has not been extensively developed. In addition to problems with collection, the samples collected by the known methods typically contain about 0.01-0.1% of the immunoglobulin found in blood serum. Because of the reduced immunoglobulin content of saliva, it has been necessary to use more accurate antigen-antibody assay methods in screening patients for disease. Parry et al., "Rational Programme for Screening Travellers for Antibodies to Hepatitis A Virus", The Lancet, June 25, 1988, have discussed such methods and have found that the more accurate IgG-capture radioimmunoassay (GACRIA) test is preferable to avoid false indications which may occur in less accurate methods. Of course, more accurate testing procedures usually require added time and expense to achieve the test results.

DISCLOSURE OF INVENTION

In order to eliminate or greatly reduce the problems inherent in antigen-antibody analysis of salival fluid, the present invention provides a method for collecting immunoglobulins from the oral cavity in a manner highly desirable for use in immunoassays. This method can be accomplished with the aid of a hypertonic solution. The method concerns placing an oral immunoglobulin collecting pad, which has been treated with a hypertonic solution, in the oral cavity to absorb a sufficient quantity of oral immunoglobulin for immunological testing. The use of the pad results in a yield of immunoglobulins greater than would be expected and can incorporate basic antigen-antibody testing techniques as a screening tool for diseases.

The hypertonic solution used in the present invention can also include additives to further provide for an optimal yield in salivary immunoglobulin content. Such additives can include compounds which maintain the correct pH, compounds which preserve the oral immunoglobulins, or compounds which inhibit the growth of organisms. The combination of such compounds provides for the collection of a salival fluid specimen which requires minimum manipulation in preparing the specimen for testing.

We have also found that the present invention can be used to collect substances other than immunoglobulins for testing. In fact, the invention has been successfully used to collect substances having molecular weights ranging from about 176 (cotinine) to about 950,000 (IgM). There is no limit to the size of the molecule which can be collected using the present invention. If the molecule can pass through the walls of the capillaries and other oral tissue, it can be collected using the present invention.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1a is a side view of the pad and pad holder of the instant invention;

Fig. 1b is top plan view of the pad and pad holder of Fig. 1;

Fig. 2 is a top view if the pad removal device according to one embodiment of the invention;

Fig. 3 is a side view of one embodiment of a container for storing the pad;

Fig. 4 is a flow diagram demonstrating how the pad is to be placed and stored in the container of Fig. 3;

Fig. 5 is a longitudinal section of another embodiment of a container for storing the pad;

Fig. 6 is a longitudinal section of the embodiment of Fig. 5 with the pad and holder shown;

Fig. 7 is a longitudinal section of a container according to a third embodiment;

Fig. 8 is a longitudinal section of a stopper for the container of Fig. 7;

Fig. 9 is an enlarged fragmentary longitudinal section showing a portion of the container of Fig. 7 and the stopper of Fig. 8; and

Fig. 10 is an enlarged fragmentary elevational view of a part of the stopper of Fig. 8.

MODES FOR CARRYING OUT THE INVENTION

The present invention is concerned with collecting oral immunoglobulins for immunological testing and other substances for testing. A treated pad is used to collect a specimen having a high concentration of immunoglobulins or the other substances. High levels of immunoglobulins from the oral cavity are considered to be concentrations in excess of 50 μg total Ig per ml. The specimen can be subjected to a basic testing technique which can be used as a tool for

screening a patient for diseases or for the presence of certain foreign substances.

Representative molecules which have been successfully collected by the use of the present invention are:

Analyte	Molecular Weight
Cotinine	176
Glucose	180
Theophylline	180
Cocaine	303
Beta2-microglobulin	11,818
Hepatitis B surface antigens	24,000
Beta-human chorionic gonadotropin	37,900
IgG -- human antibody	150,000
Total IgG (antigen not specified)	
HIV-1	
Hepatitis A	
Hepatitis B	
Rubeola (measles)	
Syphilis non-treponemal antigen	
IgA -- human antibody	160,000
Total IgA (antigen not specified)	
IgM -- human antibody	950,000
Total IgM (antigen not specified)	
Hepatitis A	
Hepatitis B	

The solution to be used in the pad of the present invention is preferably a hypertonic solution. Although a non-hypertonic solution such as water may be used, it has been found that immunoglobulin production from salivation rapidly declines in concentration using such a solution. However, the use of a hypertonic solution results in a constant production of immunoglobulin from other sources within the oral cavity, those sources not being completely understood. By using a hypertonic solution, it is possible to gain an increase of as much as 8-16 times more immunoglobulin than by using distilled water.

A hypertonic solution is a salt solution which has an ionic strength exceeding that found in blood. In general, salts used in the preparation the hypertonic solution of the present invention are present in an amount of from about 1.5% to about 5% by weight, preferably 3.5% by weight.

Salts which can be used in the preparation of the hypertonic solution include alkali metal compounds as well as alkaline earth metal compounds. Preferred salts include sodium chloride, potassium chloride, magnesium sulfate, magnesium chloride and calcium chloride. Sodium chloride is found to be the least toxic, least expensive and most palatable.

The hypertonic solution of the present invention can also include a compound or ingredient for stimulating salivation. The compounds capable of stimulating salivation are found to exhibit a sour taste. These compounds include weak organic acids. Preferred among the weak organic acids are citric acid, ascorbic acid and acetic acid. It is preferred to use citric acid and ascorbic acid at a concentration of between about 0.05% and 0.5% by weight. The preferable range for acetic acid is between about 0.5% and 3.0% by weight.

In order to minimize degradation in a collected specimen, the hypertonic solution of the present invention can include a preservative. Such a preservative can act to inhibit proteolytic enzymatic activity which can be responsible for the destruction of antibody molecules. Compounds contemplated as a preservative include anti-bacterial agents, anti-fungal agents, bacteriostatic agents, fungistatic agents, and enzyme inhibitors. In a preferred embodiment benzoic acid, sorbic acid or the salts thereof are used as anti-fungal agents. As bacteriostatic agents, salts in high concentration and compounds capable of maintaining the hypertonic solution at low pH are contemplated. Such salts

include thimerosal (or merthiolate), phenyl mercuric acetate, phenyl mercuric nitrate and sodium azide. Other preferred preservatives include preservatives which are typically used in medicines and mouthwashes. Examples include ethyl alcohol and chlorhexidine gluconate. Another class of preferred anti-microbial agents are detergents which can be used as topical germicides or in mouthwashes. An example is benzalkonium chloride. It is preferred to use these preservatives in a range of about 0.01% to about 0.2% by weight.

In the present invention, a pad containing the salts of the hypertonic solution is used to absorb saliva and mucosal secretions from the oral cavity. The pad is made of an absorbent material which can be effectively placed into the oral cavity. A plastic or carbohydrate material such as cellulose can be used as the absorbent material, but a thick, absorbent cotton paper is preferred. An example of a thick, absorbent cotton paper is product #300 manufactured by Schleicher and Schuell in Keene, New Hampshire. The pad is preferably not in the form of a foam or sponge, although foam or sponge could be used.

The pad is impregnated with the hypertonic solution by any known means. The hypertonic solution of the present invention could be applied to the pad by dipping the pad into the hypertonic solution so that the salts of the solution can be absorbed into and onto the pad, removing the pad from the solution and allowing the pad to dry. Typically, the pad is dipped into the hypertonic solution and about 1 ml of solution is absorbed. Alternatively, the hypertonic solution could be sprayed onto the pad until a sufficient amount, preferably about 1 ml is absorbed. Excess liquid is shaken off and the pad is placed into a forced air, convection drying oven at 50°C for 2 hours. After drying, there will

be formed a specially treated pad which comprises the salts of the hypertonic solution of the present invention. It is preferred that, as preservatives, such salts as benzalkonium chloride, acetyl pyridinium chloride or chlorhexidine gluconate be used in the preparation of the pad.

Most materials from which the pad is made can non-specifically bind protein. Thus, some immunoglobulins can undesirably bind to the pad and it is desired to block proteins from binding to the pad by using a blocking agent. Non-specific binding is not normally a problem in the collection of blood samples since blood contains its own blocking agent (i.e., human serum albumin).

To reduce non-specific binding in the collection of oral specimens, a blocking agent can be added to the hypertonic solution to be incorporated into the pad. A blocking agent is generally a soluble protein which is used to prevent non-specific binding of another protein to a solid surface. Compounds which can be added as blocking agents include albumin and gelatin, but any water soluble, non-toxic protein can be used as a blocking agent as long as the protein does not adversely affect antibody molecules. It is preferred to use bovine gelatin. In general, blocking agents can be added to the hypertonic solution of the present invention at a concentration of between about 0.01% and 0.2% by weight. The contents of the hypertonic solution are then incorporated into the pad as described above.

The preferred solution to be used in the preparation of the pad has the following composition:

component	conc. (wt.%)
sodium chloride	3.0%
sodium benzoate	0.1%
potassium sorbate	0.1%
bovine gelatin	0.1%

distilled water
addition of 0.1N sodium
hydroxide to increase pH
to about 6.5

To collect a substance from the oral cavity, the pad can be placed into the mouth with the aid of a holder. The pad and holder are shown in Figs. 1a and 1b. The pad holder 1 can be a hollow, plastic stick having a groove 2 at one end. The pad 3 is inserted into the groove and the holder can be manipulated to place the pad into the oral cavity, preferably between the lower gums and cheek. Placement of the pad between the lower cheek and gums facilitates absorption of secretions originating from gingival lymphoid tissue as well as secretions from submucosal lymphoid tissue and salivary gland lymphoid tissue. It is preferable that the specimen be collected by rubbing the pad back and forth between the gums and cheek for about ten seconds and then holding the pad in position for about two minutes.

After the specimen has been collected, the pad is stored in a container until immunological testing can be performed. One type of container is shown in Fig 3. It is desired that the container 4 have a centrifuge tube 5 as an outer portion of the container, and that an inner portion of the container have an inner tube 6 which mounts into the centrifuge tube. The pad is to be placed into the inner tube, and the contents therein are secured by a tube cap 7.

To place the pad in the inner tube, a pad removal device is used. The device is shown in Figs. 2 and 4. The pad removal device 8 is preferably a disk 9 which has an opening 10 through which the pad holder 1 can be inserted.

The pad can be inserted into the inner tube and prepared for storage in advance of immunological testing in the manner illustrated in Fig. 4. The tube cap 7 is removed from the container 4, and the pad 3 and holder are inserted into the

inner tube 6. The pad removal device 8 is placed over the holder. Then, the pad holder is inserted through the opening of the pad removal device and the holder pulled through the opening to remove the pad. Once the pad is placed in the inner tube, a preservative solution 11 is added. Such a preservative solution can act to inhibit enzymatic activity which can be responsible for the destruction of antibody molecules or can function as an anti-microbial agent.

Compounds contemplated for use in the inner tube as a preservative include anti-bacterial agents, anti-fungal agents, bacteriostatic agents, fungistatic agents, and enzyme inhibitors. As an antibacterial agent, it is preferred to use chlorhexidine gluconate or thimerosal.

The preservative solution to be used in the inner tube can contain one or a combination of the preservatives which can be incorporated into the hypertonic solution of the present invention. In general, the preservatives are included in a concentration which limits microbial contamination and does not adversely effect the immunoglobulins absorbed into the pad.

The preservative solution to be used in the inner tube can also contain a detergent which improves removal of antibody from the pad during centrifugation. Tween 20 (polyoxyethylene sorbitan monooleate) is a preferred detergent since it can also prevent non-specific binding of antibody to a solid surface. It is preferred to use a combination comprising about 0.01%-0.2% chlorhexidine gluconate and 0.2%-0.7% Tween 20. A combination comprising about 0.1% chlorhexidine gluconate and 0.5% Tween 20 is most preferred.

After the preservative solution is added to the inner tube, the tube cap is inserted into the container to seal in the

contents. The pad can be stored in this manner for several days until immunological testing can be initiated.

To simplify the collection and analysis of an oral specimen using the pad collection system, a kit can be provided. The kit can include a combination of the treated pad and implements used to collect and prepare the oral specimen for further immunological analysis. One preferred embodiment of the kit includes the treated pad 3 and pad holder 1; the container 4 having the inner tube 6, the outer tube 5 and the cap 7; the pad removal device 8; and the storage preservative 11.

Reference is now had to Fig. 5 wherein there is shown a prior art assembly which has been modified for use with the instant invention. The container 11, as disclosed in U.S. Patent No. 4,774,962, comprises a centrifuge tube 12 having a tapered lower end or base 13 with a downwardly tapering recess 14 in which solid matter accumulates upon centrifugation; an upper tube or container 15 having a radially outwardly projecting annular flange 16 and a cylindrical upper portion 17 at its upper end; and a plug or stopper 18. As taught by U.S. Patent No. 4,774,962, the cylindrical portion 17 and stopper 18 are of the same size and shape as the upper part of centrifuge tube 12 so they are flush with the outer surface of the centrifuge tube and the assembly presents a uniform appearance, although this feature is not important to the practice of the instant invention. In the floor 19 at the bottom of container 15 is a bore 20 to allow liquid to flow from container 15 to centrifuge tube 12 when the complete assembly is centrifuged. The container 15 is made of any suitable material such as polyethylene, glass, etc. Similarly, the stopper 18 is made of any suitable material such as polyethylene as is well-known in the art.

There are two major differences between the container 11 of Fig. 5 and the assembly of U.S. Patent No. 4,774,962. First, the prior art assembly contains a cylindrical chewable absorbent elastic body which is chewed by the user until it is sucked full of saliva. The instant invention does not utilize a body to be chewed by the user to absorb saliva although, while it would be awkward due to its size and shape, if it is impregnated with a hypertonic solution and used according to the instant invention it would fall within the scope thereof.

Second, there is a removable plug 21 in bore 20. The plug could be made of any suitable material such as wax, a plastic, etc. A suitable quantity of a preservative solution 22 is placed in the container 15. The preservative solution 22 is the same as that already described by reference to Figs. 1-4, with the same optional ingredients.

In this embodiment, the pad 3 on holder 1 is used as already described. After the pad 3 is removed from the user's mouth, stopper 18 is removed from container 15 and the pad is placed within the container 15. The holder 1 is broken off at a point outside the mouth of container 15 so it will project upwardly from the container. Then the stopper 18 is replaced. Since stopper 18 is hollow, it will securely seal the container 15 with the broken end of holder 1 extending into it as shown in Fig. 6. Holder 1 is preferably scored at a suitable location to provide for easy breaking. When the pad 3 is inserted in container 15, it will absorb at least a part of preservative solution 22.

The pad 3 is stored in container 15 until testing can be initiated. At the laboratory, the container 15, with the stopper 18 securely in place, is inverted, the seal 21 of wax or other suitable substance is removed, and the container 15 is placed in a centrifuge tube 12. The complete assembly 11

is then centrifuged whereby all the liquid, including preservative solution, saliva, etc., is drawn down through bore 20 into the centrifuge tube 12. Testing is then performed using known techniques.

As with the first embodiment, to simplify the collection and analysis of an oral specimen using the pad collection system of the second embodiment, a kit can be provided. The kit can include a combination of the treated pad and implements used to collect and prepare the oral specimen for analysis. One preferred embodiment of the kit includes the treated pad 3 and pad holder 1; the container 15 having the stopper 18 and the storage preservative 22. Optionally, a centrifuge tube 12 could be included.

In still another, preferred, embodiment, a tube having a frangible nipple instead of a wax seal is provided. Reference is made to Fig. 7 which depicts a vial generally designated by the numeral 23 having an open upper end forming an outwardly projecting annular rim or bead 24 and a lower end forming a floor 25. The wall 26 is preferably slightly tapered from the upper end to the floor 25. A nipple 27 extends downwardly from the floor 25. At the center of the inside of the floor 25 is a depression 28, preferably "V" shaped. The depression 28 causes the base end of nipple 27 to be weakened, thereby allowing the same to break off when sufficient pressure is applied. The floor 25 preferably has a slight slope at an angle α from the outside to the center. Angle α is preferably about 5°. Container 23 could be made of any suitable material such as polyethylene, glass, etc. The preferred material is a polycarbonate plastic.

Attention is now directed to Fig. 8 wherein there is shown the stopper to be used with container 23. The stopper 29 is hollow and comprises an upper shank portion 30 which is closed at its upper end, the top 31 extending radially

outwardly to define an annular flange 32 which is provided for gripping the stopper 29 to remove it from the container 23. The diameter of upper shank portion 30 is approximately the same as that of bead 24 of the container 23. The upper shank portion 30 terminates at its lower end in an annular shoulder 33. Lower shank portion 34 extends downwardly from shoulder 33.

A plurality of annular beads 35, 36, and 37 are formed on the lower shank portion 34. As can be seen in Fig. 10, the lowest bead 35 is pointed with the upper and lower faces 38 and 39, respectively, being at different angles, the preferred angles being shown by arrows 40 and 41. Upper face 38 is preferably at an angle of about 110° from the horizontal while lower face 39 is preferably at an angle of about 45° from the horizontal. As can be seen, lower face 39 tapers downwardly and inwardly from the apex of the bead 35 to the inner wall 42 of stopper 29. Intermediate bead 36 is also pointed with its upper and lower faces at different angles, preferably the same angles as upper and lower faces 38 and 40 of bead 35. Upper bead 37, however, is shaped differently. Upper bead 37 comprises a linear upper face 43, an essentially vertical linear middle face 44, and a linear lower face 45. Upper face 43 is preferably at an angle of about 70° from the horizontal as depicted by the arrows (not numbered) in Fig. 10. The corners joining upper face 43 and middle face 44, and middle face 44 and lower face 45 are preferably arcuate.

Fig. 9 shows, in enlarged form, the upper portion of container 23 within circle 46 of Fig. 7, with stopper 29 inserted in the mouth of the container 23. The outer diameter of stopper 29 at the outer edges of the beads is slightly larger than the inside diameter of the container 23. The stopper 29 is preferably made of polyethylene, although

it can be fabricated of any appropriate resilient material. When the stopper 29 is inserted in the mouth of container 23, beads 35, 36, and 37 are slightly flattened out as shown in Fig. 9 due to the slightly larger diameter of the stopper. This provides a positive seal. In addition, due to the construction of the stopper, inserting and removing the stopper is accomplished in steps, with each bead engaging or disengaging the wall of the container individually, thereby providing control over the use of the stopper.

In use, container 23 is used essentially in the same manner as container 15. The container 23 is supplied with a small amount of preservative solution 22 sealed in by stopper 29. After the pad 3 is used, stopper 29 is removed from container 23, pad 3 is inserted in the container 23, holder 1 is broken off, and stopper 29 is replaced. In the lab, the container 23, with stopper 29 in place, is inverted and frangible nipple 27 is broken off at the weak area caused by depression 28 to leave an opening at the depression 28. A centrifuge tube 12 is placed on container 23 and the necessary test is run.

As with the other embodiments, container 23 could be sold in kit form along with the impregnated pad 3 and holder 1.

The following examples show the effectiveness of the hypertonic solution of the present invention and the pad incorporating the solution of the present invention.

EXAMPLE 1

ELISA Test Data for HIV Antibody - Comparing Serum, Oral Rinse and Oral Pad Eluate

The oral rinse solution disclosed in parent application Serial No. 486,415, filed February 28, 1990, and grandparent application Serial No. 410,401, filed September 21, 1989, both of which are incorporated by reference herein in their entireties, was prepared except that the pH was adjusted to

about 6.0. A pad was prepared using the preferred pad preparation solution of the present invention. Fifteen individuals (12 seropositive, 3 seronegative) are compared for specific antibody levels in serum, rinse derived oral immunoglobulin, and pad derived oral immunoglobulin. A commercial ELISA test was used to detect HIV antibody. This test method shows the relative titer of antibody against the AIDS virus. The results show that in most cases, the pad yields higher antibody concentrations than the rinse.

PATIENT	SPECIMEN	SEROSTATUS	ELISA RESULTS (O.D. VALUE) (HIGHER NUMBER = STRONGER)
236	blood*	(+)	>2.0
	rinse**		>2.0
	pad***		>2.0
237	blood	(+)	>2.0
	rinse		1.735
	pad		>2.0
238	blood	(-)	0.077
	rinse		0.050
	pad		0.051
239	blood	(+)	>2.0
	rinse		1.423
	pad		>2.0
240	blood	(-)	0.088
	rinse		0.051
	pad		0.060
241****	blood	(-)	0.751
	rinse		0.061
	pad		0.052
242	blood	(+)	>2.0
	rinse		1.537
	pad		>2.0
243	blood	(-)	0.062
	rinse		0.045
	pad		0.046

(Example 1 continued)

PATIENT	SPECIMEN	SEROSTATUS	ELISA RESULTS (O.D. VALUE) (HIGHER NUMBER = STRONGER)
244	blood	(+)	>2.0
	rinse		>2.0
	pad		1.981
245	blood	(+)	>2.0
	rinse		1.742
	pad		>2.0
246	blood	(+)	>2.0
	rinse		1.431
	pad		>2.0
247	blood	(+)	>2.0
	rinse		1.368
	pad		>2.0
248	blood	(+)	>2.0
	rinse		1.492
	pad		1.825
249	blood	(+)	>2.0
	rinse		0.294
	pad		0.740
250	blood	(+)	>2.0
	rinse		>2.0
	pad		>2.0
251	blood	(+)	>2.0
	rinse		>2.0
	pad		>2.0

* Positive blood test results are numbers greater than 0.226.

** Positive rinse test results are numbers greater than 0.241.

*** Positive pad test results are numbers greater than 0.351.

**** This specimen shows a false positive reaction in the serum and a true negative reaction in the rinse and pad. A negative Western Blot plus additional ELISA testing confirms the false positive reaction in the serum.

EXAMPLE 2

Oral Immunoglobulin Stability Comparison
Pad Stored at 37°C With and Without Preservative

A pad was prepared using the preferred pad preparation solution of the present invention. An HIV positive individual was tested to compare immunoglobulin stability of the pad when stored in a distilled water solution and when stored in a preservative solution. The individual was tested by placing two pads in the mouth, one on each side, between the lower cheek and gum. One pad was treated with a gelatin blocking agent. The other pad was treated with the preferred pad preparation solution of the present invention. After removing the pads from the mouth of the individual, the material collected in the gelatin treated pad was eluted with a 0.5 ml solution of 0.3% Tween 20. The material collected in the pad treated with the preferred pad preparation solution was eluted with a 0.5 ml solution of 0.2% chlorhexidine gluconate and 0.3% Tween 20. The extract from each pad was divided into five aliquots. One of the aliquots from each pad was frozen immediately and labelled as time "0" specimen. The other aliquots are stored at 37°C and tested by ELISA at periods of 1, 3, 7 and 14 days. The time "0" specimen was then thawed and tested by ELISA. The results, as indicated below, show improved preservation of oral immunoglobulin when the preservative solution is used.

Number of days stored at 37°C	Preserved specimen ELISA O.D.	Unpreserved specimen ELISA O.D.
0	1.91	1.70
1	1.87	1.61
3	1.71	1.17
7	1.70	1.02
14	1.52	0.67

EXAMPLE 3

Correlation of Glucose Levels in Blood, Saliva, and sample from Pad

A pad was prepared using the preferred pad preparation solution of the present invention. Ten individuals are tested to compare glucose levels in blood, saliva, and the hypertonic solution-impregnated pad according to the preferred embodiment of the present invention. The material collected in the pad treated with the preferred pad preparation solution was eluted with a 0.5 ml solution of 0.2% chlorhexidine gluconate and 0.3% Tween 20. All glucose tests were done with a Sigma Glucose (HK 20) Quantitative, Enzymatic (Hexokinase) kit according to the manufacturer's directions. The results, as indicated below, show a significant recovery of glucose using the pad of the invention, in all cases greater than the recovery from saliva alone.

Subject No.	Plasma Glucose µg/dl	Spit Sample Glucose µg/dl	Pad Sample Glucose µg/dl	Ratio Plasma/Pad
1.	107.8	7.8	31.0	3.5
2.	84.5	4.3	13.8	6.1
3.	124.6	15.1	2.2	5.9
4.	89.2	15.1	16.4	5.4
5.	98.8	6.0	11.2	8.8
6.	94.0	23.3	32.3	2.9
7.	122.0	6.0	17.7	6.9
8.	84.5	16.0	18.5	4.6
9.	138.8	9.5	15.5	9.0
10.	121.1	363.0	450.4	----

EXAMPLE 4

A pad was prepared using the preferred pad preparation solution of the present invention. Twenty individuals are tested to compare theophylline levels in serum and the hypertonic solution-impregnated pad according to the preferred embodiment of the present invention. The material collected in the pad treated with the preferred pad preparation solution was eluted with a 0.5 ml solution of 0.2% chlorhexidine gluconate and 0.3% Tween 20. Theophylline levels were determined on an Instrument Laboratories (IL Test™) theophylline test instrument (Cat. No. 35228) according to manufacturer's directions.

Theophylline Correlation -- Serum Versus Eluate from Pad

Patient No.	Serum Theophylline (UG/ML)	Pad Eluate Theophyllin (UG/ML)
1	6.7	3.2
2	8.5	3.5
3	8.8	2.6
4	9.2	3.1
5	9.7	3.6
6	10.0	4.5
7	11.3	3.4
8	12.3	4.3
9	12.3	6.3
10	12.7	6.0
11	13.5	7.2
12	15.1	6.5
13	16.6	8.6
14	17.6	8.[illegible]
15	17.7	8.6
16	19.5	10.1
17	20.4	8.1
18	24.5	11.4
19	27.7	13.3

20 27.9 17.8

The results, as indicated above, show a significant recovery of theophylline using the pad of the invention.

EXAMPLE 5

A number of the analytes collected from the mouth by the pad of this invention were measured by means of a "dot blot" system. In this system, two microliter samples of the eluate from the pad (and serial dilutions of this eluate) are dotted on a nitrocel-lulose strip. After drying and blocking of each strip, the strips are incubated with a dilute solution of a goat or rabbit antibody specific for the analyte to be tested. After washing, the strips are incubated with a peroxidase-conjugated antibody to the goat or rabbit primary antibody. Subsequent incubation with the peroxidase substrate diaminobenzidene (dab) reveals dark brown dots at the place of the original dot of the pad eluate, if that sample contained the analyte of interest. In the case of the tests for total IgG, IgA, and IgM, the pad eluate was dotted on the strips, which was subsequently dried and blocked. This was followed by incubation with a peroxidase-conjugated goat antibody specific for the human antibody class of interest. As above, the dots are revealed by incubation with dab.

Using these systems, the following substances were detected in the pad eluate obtained from normal human subjects:

beta-2-microglobulin
albumin
transferrin
total human IgG (antigen not specified)
ceruloplasmin
total human IgA (antigen not specified)
total human IgM (antigen not specified)

EXAMPLE 6

Hepatitis A (IgM) Saliva Study

A pad was prepared using the preferred pad preparation solution of the present invention. Two groups of individuals are tested to compare hepatitis A levels in saliva and the hypertonic solution-impregnated pad according to the preferred embodiment of the present invention. The material collected in the pad treated with the preferred pad preparation solution was eluted with a 0.5 ml solution of 0.2% chlorhexidine gluconate and 0.3% Tween 20. An ELISA test was used and readings taken of optical density. The results were as follows:

Subject	Saliva Code	Pad Eluate O.D.	Interpret'n
1		0.97	pos.
2		1.02	pos.
3		0.9	pos.
4		1.1	pos.
5		1.02	pos.
6		1.1	pos.
7		0.58	pos
8		1.02	pos.
9		1.13	pos.
10		1.1	pos.
11		1.32	pos.

cutoff values for subjects 1-11 were 0.190 - 0.171 (Abbott Test)

Subject	Saliva Code	Pad Eluate O.D.	Interpret'n
	1444	0.872	pos.
	2316	0.741	pos.
	583	0.833	pos.
	392	0.81	pos.
	1297	0.752	pos.
	2723	0.626	pos.

1179	0.606	pos.
822	0.862	pos.
2232	0.861	pos.
2169	0.917	pos.
4583	0.901	pos.
3287	0.92	pos.
1108	0.788	pos.
803	0.399	pos.
2321	0.987	pos.
3852	1.05	pos.
2502	0.789	pos.
2927	1.05	pos.
4435	0.259	pos.
1822	0.812	pos.
3652	0.986	pos.
4056	0.31	pos.
2433	0.947	pos.
1877	0.953	pos.

cutoff values for subjects in second group were 0.148 - 0.177 (Abbott Test)

EXAMPLE 7

Hepatitis A (Total Antibody)

Saliva Longitudinal Study

A test for total antibody to hepatitis A was run using the same pad and procedure as in the previous Examples. The results are shown in the following table.

HEPATITIS A (TOTAL ANTIBODY)
SALIVA LONGITUDINAL STUDY

Subject	Sample No.	Date	Days From Onset	Cutoff	Blood O.D.	Interpret	Pad O.D.	Interpret
002A	002A-1	3/19/90	d16	0.526	0.089	pos.	0.255	pos.
	002A-2	4/5/90	d33	"	0.084	pos.	0.116	pos.
	002A-3	4/18/90	d46	"	0.084	pos.	0.116	pos.
	002A-4	5/2/90	d60	"	0.075	pos.	0.095	pos.
	002A-5	5/16/90	d74	"	0.08	pos.	0.083	pos.
	002A-6	6/13/90	d102	"	0.05	pos.	0.083	pos.
	002A-7	7/11/90	d130	"	0.052	pos.	0.082	pos.
003A	003A-1	4/11/90	d30	"	0.058	pos.	0.257	pos.
	003A-2	5/9/90	d58	"	0.053	pos.	0.148	pos.
	003A-3	5/23/90	d72	"	0.053	pos.	0.117	pos.
	003A-4	6/7/90	d87	"	0.046	pos.	0.191	pos.
	003A-5	6/21/90	d101	"	0.054	pos.	0.088	pos.
	003A-6	7/17/90	d127	"	0.048	pos.	0.108	pos.
	003A-7	8/14/90	d155	"	0.045	pos.	0.149	pos.
005A	005A-1	5/23/90	d38	"	0.046	pos.	0.071	pos.
	005A-2	6/7/90	d53	"	0.04	pos.	0.042	pos.
	005A-3	6/21/90	d67	"	0.048	pos.	0.053	pos.
	005A-4	7/3/90	d78	"	0.049	pos.	0.063	pos.
	005A-5	7/17/90	d83	"	0.074	pos.	0.031	pos.
	005A-6	8/14/90	d121	"	0.143	pos.	0.027	pos.
	005A-7	9/17/90	d155	"	0.144	pos.	0.032	pos.
007A	007A-1	5/31/90	d23	"	0.049	pos.	0.236	pos.
	007A-2	6/14/90	d37	"	0.043	pos.	0.303	pos.
	007A-3	6/29/90	d52	"	0.043	pos.	0.391	pos.
	007A-4	7/12/90	d65	"	0.038	pos.	0.134	pos.
	007A-5	8/23/90	d107	"	0.043	pos.	0.126	pos.
	007A-7	9/19/90	d134	"	0.039	pos.	0.134	pos.

Subject	Sample No.	Date	Days From Onset	Cutoff	Blood O.D.	Interpret	Pad O.D.	Interpret
008A	008A-1	7/17/90	d24	"	0.047	pos.	0.081	pos.
	008A-2	7/31/90	d38	"	0.049	pos.	0.082	pos.
	008A-3	8/23/90	d61	"	0.042	pos.	0.046	pos.
	008A-4	8/31/90	d89	"	0.037	pos.	0.044	pos.
	008A-5	9/13/90	d82	"	0.05	pos.	0.083	pos.
	008A-6	10/11/90	d110	"	0.041	pos.	0.031	pos.
	008A-7	11/12/90	d142	"	0.048	pos.	0.028	pos.
006A	0906A-1	5/29/90	d46	"	0.041	pos.	0.148	pos.
010A	010A-1	6/22/80	d17	"	0.057	pos.	0.155	pos.

THIS IS AN ABBOTT COMPETITIVE TEST FOR TOTAL ANTIBODY TO HEP A
POSITIVE CONTROL = 0.094; NEGATIVE CONTROL = 0.959

EXAMPLE 8

Hepatitis B Surface Antigen (HBsAg)

Hepatitis B surface antigen was detected in saliva samples collected with the pad using the Auszyme™ test kit made by Abbott, Inc. The mean negative control value was -0.003 and the mean positive control was 2.091. The cutoff value was calculated to be 0.047. The results are as follows:

	Abbott Auszyme
sample #	HBsAG (pad)
001-B	0.891 (+)
30-M	3.352 (+)
38-M	3.352 (+)
68-M	1.568 (+)

EXAMPLE 8

IgM Antibodies to Hepatitis B Core Antigen (HBcAg)

IgM antibodies to Hepatitis B core antigen were detected in saliva samples collected with the Pad using the Corzyme-M™ test kit made by Abbott, Inc. The mean negative control value was 0.058 and the mean positive control was 1.023. The cutoff value was calculated to be 0.314.

	Abbott Corzyme-M
sample #	IgM anti-HBc (pad)
30-M	1.492 (+)
38-M	2.206 (+)

EXAMPLE 9

IgG antibodies to Hepatitis B core antigen (HBcAg)

IgG antibodies to Hepatitis B core antigen were detected in saliva samples collected with the pad using the OraQuick

assay of the assignee hereof. The OraQuick assay is an ELISA-type assay that utilizes recombinant hepatitis B core antigen and a goat antibody specific for human IgG antibody. Positive controls demonstrated a color with intensity in the range of "+" to "+++". Negative controls were colorless ("-").

Epitope OraQuick	
sample #	IgG anti-HBc (pad)
4-M	+
8-M	++
32-M	++
38-M	++
43-M	+
69-M	+

EXAMPLE 10

Feasibility Study Measles IgG

A feasibility study using the pad of the invention to collect samples for measuring on measles IgG was conducted. A Pharmacia IgG ELISA kit was used for the tests.

KIT CONTROLS	O.D. 492
High positive	1.395
Low positive	0.958
Low positive	0.948
Neg control	0.060
Neg control	0.044
Background	0.050

PATIENT #	PAD OD	RESULTS
216	0.729	+
219	0.744	+
221	0.735	+
222	0.673	+
223	0.111	+
227	0.169	+
230	0.176	+
237	0.276	+
238	0.561	+
248	0.535	+
250	0.163	+
252	0.112	+
254	0.178	+
255	0.425	+

14 of 14 samples positive 100% correlation

calculated cutoff value = mean of the negatives x 2 = 0.104
(0.052 x 2 = 0.104)

EXAMPLE 11

Detection of Antibody to Syphilis Non-Treponemal Antibodies in Pad Samples

Positive samples were identified among samples from S.F. General Hospital by testing sera on the RPR Card Test from Becton-Dickinson. An ELISA-type membrane assay ("rapid assay") was developed using the cardiolipinphosphatidyl choline-cholesterol mixture spotted on an Immobilon membrane. This was followed by blocking and 1) exposure to serum or saliva samples, 2) washing, 3) exposure to peroxidase conjugated-goat antibody to human IgG, 4) washing, 5) exposure to TMB chromagen. Results were as follows:

Sample #	PPR Card Test	Serum Result on ELISA	Saliva (OraSure) Result on ELISA
169	pos.	pos.	pos.
196	pos.	pos.	pos.
control	neg.	neg.	neg.

EXAMPLE 12

β-HCG Levels in Pad Saliva

	HCG Concentration* mIU/ml
Negative Controls:	
"1"	0.9
"2"	0.4
Pregnant Patients:	
"A"	3.9
"B"	15.2
"C"	2.1

* Determined using the Abbott β-HCG 15/15 Enzyme Immunoassay kit. The Point-to-Point quantitative procedure was followed.

Although many embodiments of the present invention are disclosed, it is to be understood that these embodiments are not limiting. For example, many components can be incorporated into the hypertonic solution of the present invention. The disclosed components represent specific examples which are capable of yielding an increased immunoglobulin concentration in oral specimens. Of course, any list of components cannot be exhaustive and alternatives can be predicted within the scope of the contemplated invention.

WHAT IS CLAIMED IS:

1. A method of collecting substances from an oral cavity for testing comprising the steps of:

(a) inserting an absorbent pad impregnated with the salts of a hypertonic solution, wherein the salts of the hypertonic solution are in an effective concentration in the pad to recover a high concentration of said substances, into the oral cavity,

(b) removing the pad from the oral cavity, and

(c) preserving the pad for subsequent removal of the collected substances from the pad for analysis testing.

2. The method of claim 1, wherein the hypertonic solution includes alkali metal salts or alkaline earth metal salts.

3. The method of claim 1, wherein the pad is stored in a container when the pad is removed from the oral cavity.

4. The method of claim 3, wherein the container comprises an open upper end sealed with a removable stopper and a lower end having an opening communicating the interior of the container with the outside, said opening being selectively sealed during storage of said pad and unsealed for said removal of said collected substances for subsequent testing.

5. The method of claim 4, wherein said opening is selectively sealed by a removable wax seal.

6. The method of claim 4, wherein said opening is selectively sealed by a removable resilient seal.

7. The method of claim 4, wherein said opening is selectively sealed by a frangible nipple.

8. The method of claim 3, wherein the container includes a preservative solution.

9. The method of claim 8, wherein the preservative solution includes chlorhexadine gluconate.

10. The method of claim 1, wherein the hypertonic solution includes a salival stimulating agent.

11. The method of claim 1, wherein said substances are analytes having a molecular weight from about 176 to about 950,000.

12. The method of claim 11, wherein said analytes are selected from the group consisting of cotinine, glucose, theophylline, cocaine, beta 2-microglobulin, Hepatitis B surface antigens, beta-human chorionic gonadotropin, and immunoglobulins, and mixtures thereof.

13. The method of claim 12, wherein said substances are immunoglobulins.

14. The method of claim 13, wherein said immunoglobulins are selected from the group consisting of IgG, IgA, and IgM.

15. The method of claim 14, wherein said immunoglobulins are antibodies against at least one of a member of the group consisting of HIV-1, hepatitis A, hepatitis B, rubeola, and syphillis non-treponemal antigen.

16. The method of claim 13, wherein the hypertonic solution includes a blocking agent.

17. The method of claim 16, wherein the blocking agent is albumin or gelatin.

18. The method of claim 1, wherein said analysis is by immunological testing.

19. The method of claim 18, wherein said immunological test is an ELISA test.

20. A pad for collecting substances from an oral cavity for testing comprising an absorbent material impregnated with the salts of a hypertonic solution, wherein said salts of a hypertonic solution are in an effective concentration in the pad to recover a high concentration of said substances.

21. The pad of claim 20, wherein said salts of a hypertonic solution are impregnated into the pad by:

(a) dipping the pad into the hypertonic solution; and

(b) drying the pad;

(c) wherein the hypertonic solution is in an effective concentration to provide an effective concentration of the salts thereof in the pad to recover a high concentration of said substances.

22. The pad of claim 20, wherein said salts of a hypertonic solution are impregnated into the pad by:

(a) spraying the pad with the hypertonic solution and;

(b) drying the pad;

(c) wherein the hypertonic solution is in an effective concentration to provide an effective concentration of the salts thereof in the pad to recover a high concentration of said substances.

23. The oral immunoglobulin collecting pad of claim 20, wherein the hypertonic solution includes alkali metal salts or alkaline earth metal salts.

24. The oral immunoglobulin collecting pad of claim 20, wherein the pad is made of a carbohydrate material.

25. The pad of claim 20, wherein said substances are immunoglobulins.

26. The oral immunoglobulin collecting pad of claim 25, further comprising a blocking agent.

27. The pad of claim 26, wherein the blocking agent is albumin or gelatin.

28. The pad of claim 25, wherein the pad is made of a carbohydrate material.

29. The pad of claim 25, wherein the hypertonic solution includes alkali metal salts or alkaline earth metal salts.

30. The pad of claim 25, wherein the hypertonic solution includes a preservative.

31. The pad of claim 30, wherein the preservative includes chlorhexadine gluconate.

32. The pad of claim 25, wherein the hypertonic solution includes a salival stimulating agent.

33. A container for storing collected substances for subsequent testing comprising an open upper end adapted to be sealed with a removable stopper and a lower end having an opening communicating the interior of the container with the outside, said opening being selectively sealed during storage of said substances and unsealed for removal of said collected substances for subsequent testing.

34. The container of claim 33, wherein said opening is selectively sealed by a removable wax seal.

35. The container of claim 33, wherein said opening is selectively sealed by a removable resilient seal.

36. The container of claim 33, wherein said opening is selectively sealed by a frangible nipple.

37. A method for recovering for testing substances collected from an oral cavity in an absorbent pad impregnated with the salts of a hypertonic solution, wherein the pad is stored in a container after removal from the oral cavity, wherein said container comprises an open upper end sealed with a removable stopper and a lower end having an opening communicating the interior of the container with the outside, said opening having a removable seal thereon during storage of said pad, said method comprising eluting said substances with a solvent or diluent therefor within said container, removing said seal, placing said container in a centrifuge tube with the lower end of said container within the centrifuge tube to form an assembly, and centrifuging said assembly to thereby draw said solvent or diluent containing said substances into said centrifuge tube.

38. The method of claim 37, wherein said seal is a removable wax seal.

39. The method of claim 37, wherein said seal is a removable resilient seal.

40. The method of claim 37, wherein said seal is a frangible nipple.

41. A kit for collecting and storing substances from an oral cavity for subsequent testing comprising a pad treated with a hypertonic solution; and a container for storing the pad; said container comprising an open upper end adapted to be sealed with a removable stopper and a lower end having an opening communicating the interior of the container with the outside, said opening being selectively sealed during storage of said substances and unsealed for removal of said collected substances for subsequent testing.

42. The kit of claim 41, further comprising a removable stopper.

43. The kit of claim 41, wherein said opening is selectively sealed by a removable wax seal.

44. The kit of claim 41, wherein said opening is selectively sealed by a removable resilient seal.

45. The kit of claim 41, wherein said opening is selectively sealed by a frangible nipple.

46. The kit of claim 41, wherein the container includes a preservative solution.

47. The Kit of claim 46, wherein the preservative solution includes chlorhexadine gluconate.

48. The kit of claim 41, wherein the hypertonic solution includes a salival stimulating agent.

FIG. 1a

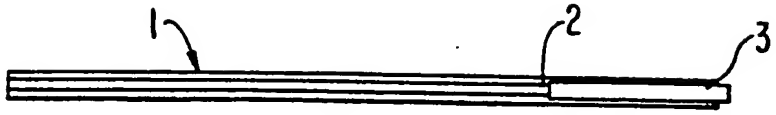


FIG. 1b

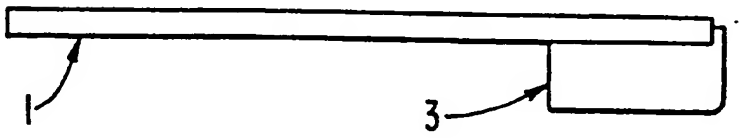


FIG. 2

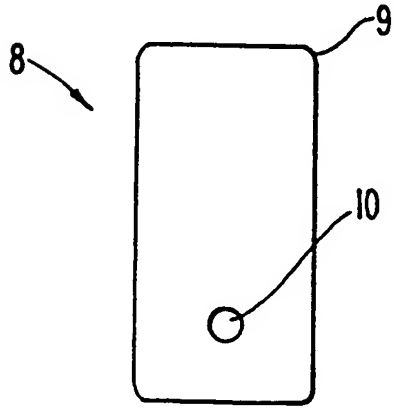


FIG. 3

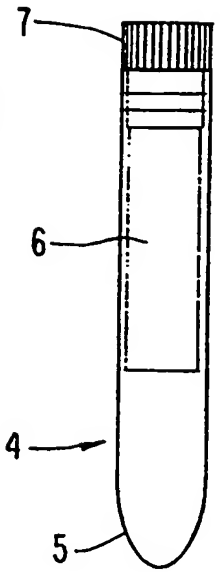


FIG. 4

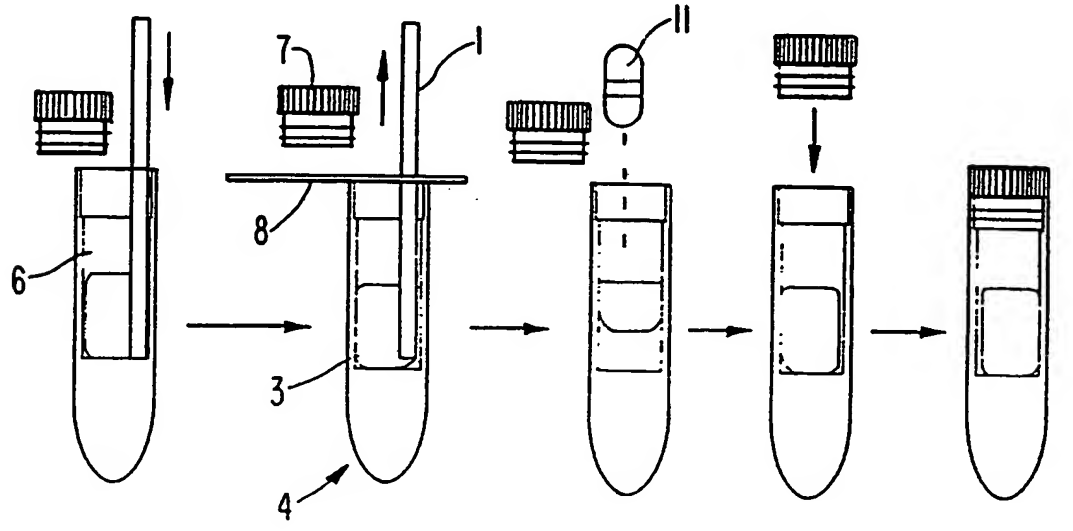


FIG. 5

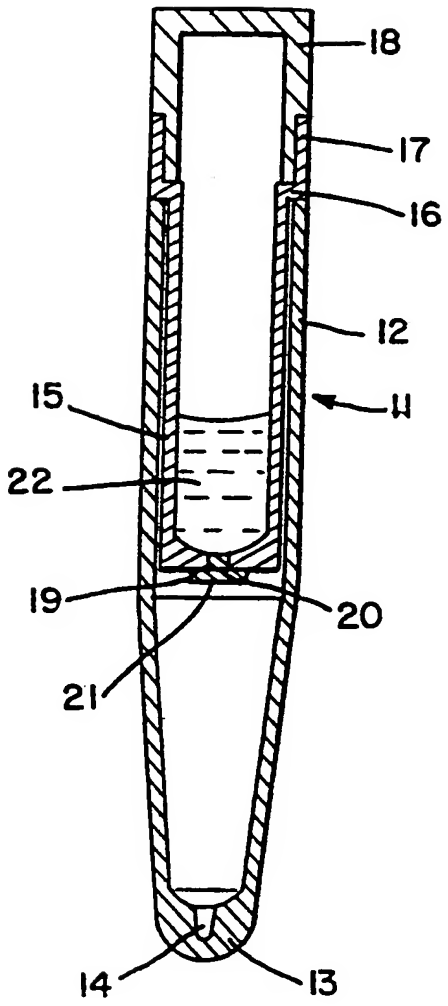
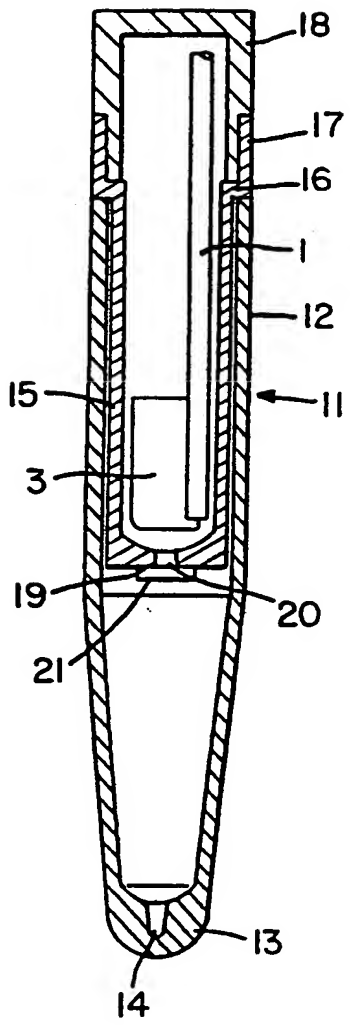


FIG. 6



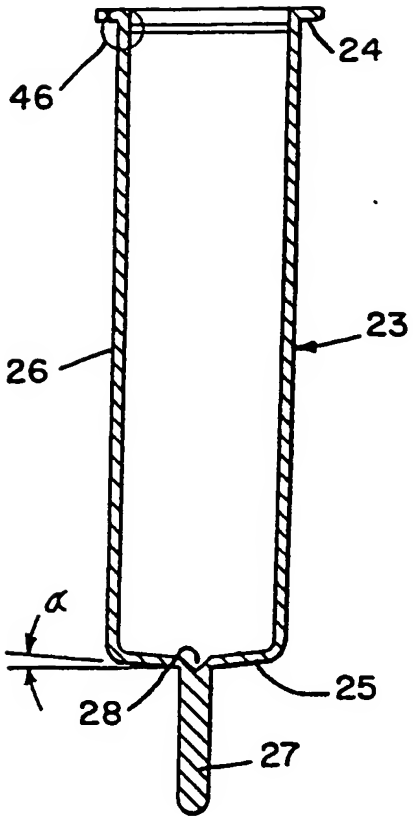


FIG. 7

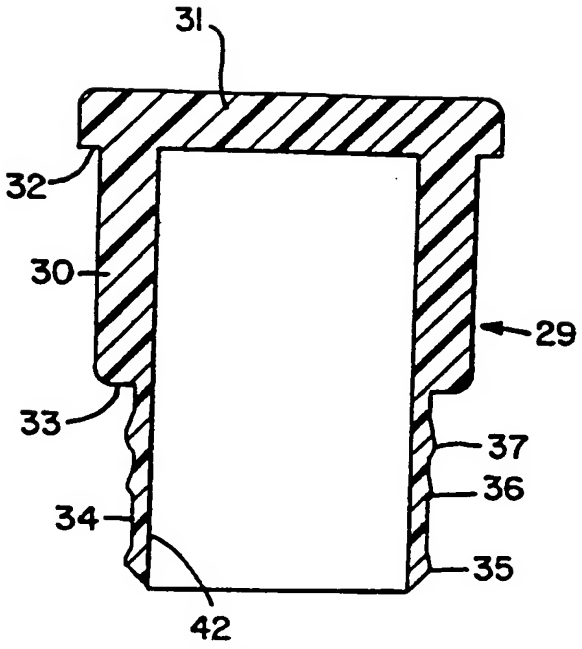


FIG. 8

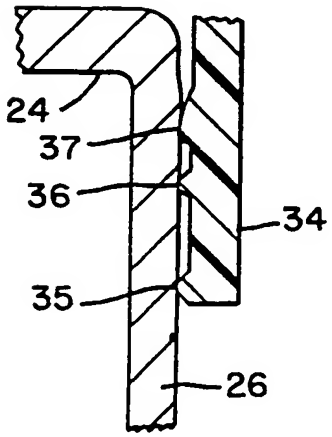


FIG. 9

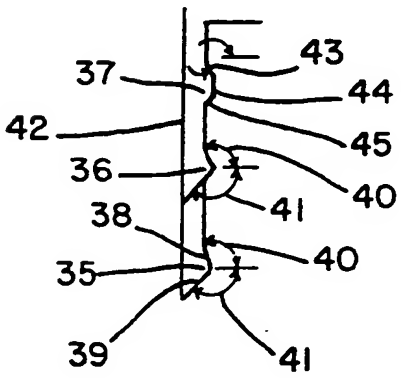


FIG. 10